



*Official Journal of the  
Malaysian Medical Association*

# *The Medical Journal of Malaysia*

**Volume: 80**

**Issue No: 4**

**July 2025**



# MJM

*Official Journal of the  
Malaysian Medical Association*

Volume 80 Number 4 July 2025

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PP 2121/01/2013 (031329)

MCI (P) 124/1/91

ISSN 0300-5283

The Medical Journal of Malaysia is published six times a year.  
MJM is published bimonthly ie. January, March, May, July, September and November.

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Printed by: Digital Perspective Sdn. Bhd.  
42-1, Level 1, Plaza Sinar, Taman Sri Sinar, 51200 Kuala Lumpur. Tel: 03-6272 3767  
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## Acknowledgements:

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## Example references Journals:

### Standard Journal Article

Rampal L and Liew BS. Coronavirus disease (COVID-19) pandemic. Med J Malaysia 2020; 75(2): 95-7.

Rampal L, Liew BS, Choolani M, Ganasegeran K, Pramanick A, Vallibhakara SA, et al. Battling COVID-19 pandemic waves in six South-East Asian countries: A real-time consensus review. Med J Malaysia 2020; 75(6): 613-25.

NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: a pooled analysis of 1201 population-representative studies with 104 million participants. Lancet 2021; 11; 398(10304): 957-80.

## Books and Other Monographs:

### Personal Author(s)

Goodman NW, Edwards MB. 2014. Medical Writing: A Prescription for Clarity. 4 th Edition. Cambridge University Press.

### Chapter in Book

McFarland D, Holland JC. Distress, adjustments, and anxiety disorders. In: Watson M, Kissane D, Editors. Management of clinical depression and anxiety. Oxford University Press; 2017: 1-22.

### Corporate Author

World Health Organization, Geneva. 2019. WHO Study Group on Tobacco Product Regulation. Report on the scientific basis of tobacco product regulation: seventh report of a WHO study group. WHO Technical Report Series, No. 1015.

NCD Risk Factor Collaboration (NCD-RisC). Rising rural body-mass index is the main driver of the global obesity epidemic in adults. Nature 2019; 569: 260-64.

World Health Organization. Novel Coronavirus (2019-nCoV) Situation Report 85, April 14, 2020. [cited April 2020] Accessed from: <https://www.who.int/docs/defaultsource/coronaviruse/situationreports/20200414-sitrep-85-covid-19>.

## Online articles

**Webpage:** Webpage are referenced with their URL and access date, and as much other information as is available. Cited date is important as webpage can be updated and URLs change. The "cited" should contain the month and year accessed.

Ministry of Health Malaysia. Press Release: Status of preparedness and response by the ministry of health in and event of outbreak of Ebola in Malaysia 2014 [cited Dec 2014]. Available from: [http://www.moh.gov.my/english.php/database\\_stores/store\\_view\\_page/21/437](http://www.moh.gov.my/english.php/database_stores/store_view_page/21/437).

## Other Articles:

### Newspaper Article

Panirchellvum V. 'No outdoor activities if weather too hot'. the Sun. 2016; March 18: 9(col. 1-3).

### Magazine Article

Rampal L. World No Tobacco Day 2021 -Tobacco Control in Malaysia. Berita MMA. 2021; May: 21-22.

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All original papers which are accepted for publication by the MJM, will be considered for the 'Best Paper Award' for the year of publication. No award will be made for any particular year if none of the submitted papers are judged to be of suitable quality.

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# Moving From Tobacco Control to Tobacco Endgame in Malaysia

Lekhraj Rampal<sup>1,2</sup>

<sup>1</sup>Advisor, Medical Journal of Malaysia (Editor in Chief 2014- 2024), <sup>2</sup>Advisor, Action on Smoking and Health Committee, Malaysian Medical Association (1999 – 2008; 2013 – 2022, and Advisor 2022 – 2025)

## BACKGROUND

The tobacco epidemic is one of the biggest public health threats worldwide. Tobacco remains one of the few legally available products that cause significant harm not only to users but also to those exposed to second-hand smoke. Tobacco consumption, regardless of form or disguise, cigarettes, smokeless tobacco, water pipes commonly known as shishas, narghiles, or hubble-bubbles, is highly addictive due to nicotine in tobacco products and is deadly. Each year, tobacco use kills more than eight million people, including an estimated 1.3 million non-smokers who are exposed to second-hand smoke. Its use is a significant risk factor for cardiovascular, respiratory diseases, cancer, and many other debilitating health conditions. Beyond health effects, the socio-economic costs to individuals, families, businesses, governments, and health systems are substantial.

### Historical ‘No Smoking Day’ on 5th November 1986.

The Action on Smoking and Health Committee of the Malaysian Medical Association (ASH, MMA), in collaboration with the MMA Public Health Society (MMA PHS), demonstrated strong leadership in organising the inauguration of the first Malaysian ‘No Smoking Day’ on 5 November 1986 (<https://www.e-mjm.org/2015/v70s1/mjm-sept-suppl-2069.html>). Tun Dr Siti Hasmah officiated the landmark event. It was organised by ASH MMA, in collaboration with the PHS, the National Heart Association, the National Cancer Society, the Ministry of Health, and multiple sectors, including schools, hotels, and banks. Notably, ASH MMA conducted No Smoking Day without any fund from the Government. Despite limited resources, it achieved remarkable reach and success; 80% awareness was recorded across eight evaluation sites nationwide. Media coverage was extensive, with the campaign featured on the

front page of major newspapers and shared on the radio and television. The success and public reception were such that ASH MMA received requests to expand the initiative into a whole week. Significantly, this national campaign preceded the global call for tobacco control awareness by the World Health Organization (WHO). The World Health Assembly (WHA) adopted Resolution WHA40.38 later. In 1987, the WHA passed Resolution WHA40.38 in 1987, declaring 7 April 1988 to be the first “World No-Smoking Day.” This was followed by resolution WHA42.19, which was passed in 1988, calling for the World No Tobacco Day celebration every year on 31 May. Since then, the WHO has supported World No Tobacco Day (WNTD) annually, linking it to a different tobacco-related theme each year.

The first ‘No Smoking Week’ was held from 1 to 7 November 1987. With the theme ‘Help a Friend Quit Smoking.’ The campaign unfolded during a sensitive period, as Operation Lalang, was proclaimed under the Internal Security Act (ISA), was declared on 27 October 1987, under the Internal Security Act (ISA). The Operation Lalang resulted in a sweeping crackdown on opposition parties and Non-Governmental Organisations (NGOs), with more than a hundred Malaysians detained without trial. The prevailing climate of fear and uncertainty had a chilling effect on the civil society. Despite the tension, the campaign proceeded. Due to safety concerns, the Chairman of ASH MMA called from Ipoh and said that he would not be attending the launch, entrusting Dr. Lekhraj Rampal



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(as the Secretary of ASH MMA and Chairman of the Public Health Society) to lead the event. A pivotal moment occurred during the launch when Datuk K Pathmanaban publicly declared his intention to quit smoking during the press interview. He not only followed through but also permanently ceased smoking, garnering positive publicity for himself and the Malaysian Medical Association. Throughout this period, MMA remained a steadfast supporter and sponsor of all ASH MMA activities. ASH MMA remained steadfast in its activities and initiatives, reinforcing its commitment to tobacco control despite external pressure. Over the decades, ASH MMA continued to be at the forefront of tobacco advocacy in Malaysia. In collaboration with key stakeholders, including the Ministry of Health (MOH), the Ministry of Education (MOE), Malaysian Council for Tobacco Control (MCTC), Universiti Putra Malaysia (UPM), NGOs, schools, and banks the following were achieved in 1983 National workshop on smoking control; 1986 National No Smoking Day; 1987 National No Smoking Week; 1988 National No Smoking Week; 1989 National No Smoking Week; 1990 National No Smoking Week; 1991 National No Smoking Week; 1992 National No Smoking Week, the National No Smoking Week in 1993; 1998 International Quit and Win; 1999 National No Smoking Day; 2000 International Quit and Win; 2002 International Quit and Win; 2004 International Quit and Win; 2005 National No Smoking Day; 2006 International Quit and Win; 2016 World No Smoking Day; 2017 World No Smoking Day; 2018 World No Smoking Day; 2019 World No Smoking Day; 2020 World No Smoking Day; 2022 World No Smoking Day, and the 2023 World No Smoking Day. A landmark policy change attributed to ASH MMA's advocacy was the ban on smoking aboard Malaysian Airlines (MAS) flights implemented on January 1, 1996. Initially covering all domestic flights and most international routes. This sets a precedent for smoke-free transport policies in the region. Central to these efforts has been Professor Datuk Dr Lekharaj Rampal, a committed tobacco control advocate for over 30 years. His roles include Secretary, ASH MMA (1982-1993); Chairman, ASH MMA (1993-1994, 1999-2008, 2013-2022); Advisor (2022-2025); and Deputy President, MCTC (2016-2018); and President, MCTC (2018-2022). MCTC comprises more than 40 NGOs related to Tobacco Control. ASH MMA has played a transformative role in Malaysia's Tobacco control movement, not only in public health education but more significantly through its relentless pursuit of policy reform. Recognising that health education alone could not withstand the aggressive tactics of the global tobacco industry. ASH MMA, under the leadership of Dr. Rampal, championed structural and legislative solutions, such as tax increases, comprehensive bans on tobacco advertising, plain packaging, smoke-free public spaces, and a ban on vaping. ASH MMA has consistently been ahead of its time, proposing policies long before they became mainstream discourse. Star with Example, Dr. Rampal advocated for raising the smoking age to 21, long before it became part of our legislative consideration. Similarly, ASH MMA lobbied for the criminalisation of illegal cigarette trade as early as the 1990s. Today, many of those ideas have found their place within the framework of 'The Control of Smoking Products for Public Health Act 2024', as a testament to this vision and consistency. Malaysia's involvement in the International

Quit and Win (IQSW) further underscored its commitment to tobacco control.

Recognised for his experience and leadership, Dr. Rampal was invited by Prof. Dr. Pekkias Puska, Chairman of the IQSW campaign in Malaysia, as part of the global initiative. IQSW was the most significant practical international initiative for smoking cessation. The number of countries and smokers in the IQSW Smoking Cessation contest increased from 13 countries in 1994 to 25 in 1996 to 50 countries in 1998, 70 countries in 2000, and 76 countries in 2002. In 2004, 71 countries with 700,000 smokers took part, compared to 13 countries with 60,000 smokers in 1994. In Malaysia, the IQSW Smoking Cessation contest campaigns were held in collaboration with the MOH in 1998, 2000, 2002, and 2004. It was held together with World No Tobacco Day. The number of smokers participated in the IQSW Smoking Cessation contest increased from 945 in 1998 to 1,155 people in 2000, and to over 2,000 smokers in 2002 from all parts of the country. Several activities were carried out, including talks, exhibitions, and National Art competitions. A large number of participants agreed to give up smoking for at least one month. The art competition was also a considerable success. There was also broad coverage by the press, radio, and TV. For several weeks, feature stories and letters commented on smoking and its problems. The World No Tobacco Day and the prize-giving ceremony for the winners of IQSW in 2004 and 2006 were held at UPM. More than 800 people, including the Vice Chancellor and staff, attended each of the functions. In 2004, 2006, 2016, 2017, and 2018, the World No Tobacco Day and the prize-giving ceremony for the winners of the Art competition were held at UPM. There was good mass media coverage on the World No Tobacco Day and the IQSW contest. A significant outcome of ASH MMA's persistent advocacy materialised in 2000, when the MOH first established the "Quit Smoking Clinic" following ASH MMA's recommendation, making smoking cessation services accessible to everyone. Since then, the MOH has established a Tobacco Control Unit and the Framework Convention for Tobacco Control (FCTC) Secretariat. These developments led to the establishment of more than 300 quit-smoking clinics and the integration of cessation services in 32 hospitals across the nation. These clinics offer counselling, support, and pharmacological treatments, such as nicotine replacement therapy (NRT). These initiatives play a vital role in helping individuals stop smoking by providing non-pharmacological and pharmacological interventions.

#### **The Tak Nak Campaign 2004**

The Tak Nak Campaign, launched in 2004 by Tun Abdullah bin Haji Ahmad Badawi, the then Prime Minister of Malaysia, marked another significant milestone in the country's tobacco control efforts. The launch event drew over 2,500 participants and garnered extensive media coverage.

It was an enormous success. For the launch, over 2,500 participants attended and received extensive media coverage in newspapers and on TV channels. The campaign was branded with the slogan "Tak nak", which translates to "Say no to Smoking". The campaign was branded with the slogan "Tak nak", which translates to "Say no to Smoking". It was designed to instil a strong anti-smoking message, particularly



Prof Datuk Dr Lekhraj Rampal President MCTC, briefing YB Datuk Seri Dr Haji Dzulkefly, Minister of Health during the historical MCTC meeting at Parliament House in 2018.

among young people. During the first year, the Tak Nak Campaign was widely regarded as successful in achieving its stated goals, generating substantial public support and engagement. Critics and those with a vested interest may say it was a failure. The then-new Minister of Health, Malaysia, expressed the view that the campaign was not successful. This led to a loss of focus on its continuation at a national level by the MOH. ASH MMA, realising the impact of the campaign, however, continued to use the slogan 'Tak Nak' in their campaigns.

#### Call for ban on e-cigarettes, vapes, and shishas

Between May 2015 and March 2016, several meetings were organised by the MOH related to the problem of e-cigarettes, vapes, and shishas. Prof. Dr. Lekhraj Rampal, as Chairman of ASH MMA, recommended to the Government to ban e-cigarettes, vapes, and shisha. The Director General and Deputy Director General (Public Health) of MOH had also recommended a ban on e-cigarettes, vapes, and shishas. The Minister of Health recommended to the Cabinet of Malaysia to ban e-cigarettes, vapes and shisha. However, the Cabinet of Malaysia decided instead to regulate e-cigarettes, vapes, and shishas. MMA and MCTC, since then, have consistently recommended to the Government to ban e-cigarettes, Vapes, and Shisha. His Royal Highness Sultan Ibrahim ibni Almarhum Sultan Iskandar, the current King of Malaysia had strongly advocated for a ban on the sale of e-cigarettes and vapes and ordered a ban on their sale in the State of Johor. Several NGOs and personalities from MCTC participated in television and mass media programs to discuss why the Government should ban it. In recent years, several states in Malaysia have banned the sale of e-cigarettes, vapes, & Shisha.

#### Historical World No Tobacco Day 2018

World No Tobacco Day 2018 was held on 31 May 2018 at the main hall, Faculty of Medicine and Health Sciences, UPM, marking a significant turning point in Malaysia's tobacco control landscape. Themed 'tobacco breaks Hearts', the event was historical in that it received direct support of Datuk Seri Haji Dr. Dzulkefly Bin Ahmad, Minister of Health. This was the first Minister of Health in the last four decades that a Minister of Health had positively and promptly responded to the invitation of ASH MMA to launch World No Tobacco

Day. During this event, Prof Dr Rampal, in his capacity as President of MCTC and Chairman of ASH MMA, presented a set of strategic recommendations to the Minister on behalf of MCTC and ASH MMA. These included expedite the tabling of a comprehensive Tobacco Control Act in Parliament, raising the legal age for sale and purchase of tobacco and tobacco products from 18 years to 21 years, Increase the tax on tobacco and tobacco products to at least 70% of the retail price as per the World Bank's recommendation, channel the Tax (Tax Haram) on Tobacco products in to for prevention programs and for Smokers who want to quit, enforce a ban on display of tobacco and tobacco products at the point of sale locations. Additional recommendations include ratifying the Protocol to Eliminate Illicit Trade in Tobacco Products adopted by the Conference of the Parties to the WHO FCTC, which will help to eliminate illicit trade in tobacco products by all SEA countries, increasing the penalties for offences related to illegal trade in tobacco and tobacco products, expedite in the implementation of licensing of tobacco and tobacco products, and enforce no smoking at all workplace environments as provided for in the Occupational Safety and Health Act 1994 and government service circulars. This advocacy momentum continued with a follow-up meeting with the Minister of Health in the meeting room at the Parliament House.

Under the leadership of Datuk Seri Haji Dr Dzulkefly, one of the most impactful policies was a ban on smoking at all restaurants and other eateries, including open-air hawker stalls, which went into effect on 1 January 2019. The Minister also assured Prof. Dr. Lekhraj Rampal at the WHO FCTC steering committee meeting that the new Tobacco Act will be passed in Parliament by the end of 2019. However, this did not occur due to the political instability and frequent changes in government and the Minister of Health Datuk Seri Haji Dr Dzulkefly (from May 2018 to February 2020), Dato' Sri Dr. Adham (from 10 March 2020 to 16 August 2021), YB Mr Khairy Jamaluddin, Minister of Health (from August 2021 to November 2022) and Datuk Seri Haji Dr Dzulkefly (second term since 12 December 2023). During the period 2019/2020, Tobacco Control activities were further affected due to the COVID-19 Outbreak. On 13 January 2022, the President of MCTC, along with his committee, was invited to a meeting with YB Khairy Jamaluddin, Minister of Health, the Director General of Health, and Senior staff of the FCTC in Putrajaya. The President of MCTC briefed the Minister on the History of Tobacco Control in Malaysia and the MCTC's recommendations to the Government of Malaysia, which had been presented to the earlier Minister of Health. YB Mr Khairy Jamaluddin was positive, but his tenure as a Minister was too brief to be able to implement the substantial tobacco control policy reforms. The World No Tobacco Day 2022, with the Theme 'Towards A Smoke-Free Generation,' was jointly launched by the Minister of Health, YB. Khairy Jamaluddin and YB. Senator Ustaz Datuk Haji Idris Ahmad, Minister in the Prime Minister's Department for Religious Affairs, on 22 April 2022. The campaign aimed to empower and support tobacco users attending religious places, on their quitting journey. This was followed by another World No Tobacco Day 2022 event launched by YB. Khairy Jamaluddin, Minister of Health, and Datuk Dr Mohd Radzi bin Md. Jidin, Senior Minister of Education, Malaysia. The

objectives were to discourage youth from taking up smoking and those who had started smoking to quit, increase awareness among the community about the hazards of tobacco, and promote the community's understanding of the importance of a tobacco-free environment. The activity targeted three categories: Primary, Secondary schools, and the Public. ASH MMA organised the National Art (Poster) Competition in collaboration with the Ministry of Education (MOE). There was a total of 9,659 entries for the National Art Competition.

The World No Tobacco Day 2023 was held at Dewan Hang Tuah, Malacca. It was organised by ASH MMA in collaboration with MCTC and NCSM. The event was attended by approximately 500 people: VIP guests, teachers, students, winners of the art competition, and their parents. A series of webinars and seminars on several topics related to smoking and health were held.

### Malaysia Book of Records

ASH MMA also organised the National Art Competition in collaboration with the Ministry of Education. The competition entered the Malaysia Book of Records for the Most Anti-Smoking Art Poster Competition. There were 24,248 entries. The certificate was presented at the Malaysia Book of Records Office in Kuala Lumpur on 23 May 2023. The earlier record was 13,300 participants in 2018, organised by ASH MMA in collaboration with the Faculty of Medicine and Health Sciences, UPM.

The Control of Smoking Products for Public Health Act 2024 The close collaboration between the Minister and the MOH, ASM MMA, and MCTC has become even more focused in recent years, as Malaysia began drafting what became Act 852. It was necessary, bold, and disruptive, and it was needed. It has become a comprehensive legislation covering conventional cigarettes, e-cigarettes, vape products, and future nicotine devices, which was conceived as part of a broader strategy to push Malaysia toward a Tobacco End Game. And few people understood this better than the Minister of Health, MCTC, and MMA. Professor Rampal became a pillar of support, technically, morally, and politically, helping build bridges between stakeholders, guiding scientific rationale, and amplifying the public health urgency. Through his leadership, the message was clear: public health must be governed by principle, not expediency alone. MCTC under the leadership of Prof. Rampal's advocacy for Act 852, was not about enacting another law; it was about enshrining a legacy of protection for future generations. On 1 October 2024, the Control of Smoking Products for Public Health Act 2024 (ACT 852), came into force. It is the primary legislation governing tobacco control in Malaysia. It regulates, among other things, smoke-free environments; tobacco advertising, promotion, and sponsorship; tobacco packaging and labelling; sales restrictions, content, and emissions regulation; and disclosure. It regulates all smoking products, including tobacco products, smoking substances, and substitute tobacco products. E-cigarettes and heated tobacco products, as well as traditional tobacco products are also regulated under this Act. The regulations that have been issued under the Act include Control of Smoking Products for Public

Health (Registration of Tobacco Product, Smoking Substance and Substitute Tobacco Product) Regulations 2024, the Control of Smoking Products for Public Health (Packaging and Labelling) Regulations 2024, the Control of Smoking Products for Public Health (Warning Sign of Prohibition of Smoking) Regulations 2024, Control of Smoking Products for Public Health (Control of Sale) Regulations 2024, and the Control of Smoking Products for Public Health (Declaration of Non-Smoking Area or Place) Order 2024. This Act and its implementation regulations replace the earlier Control of Tobacco Product Regulations 2004 (and amendments) that were issued under the Food Act of 1983.

The passing of Act 852 is a milestone, but it was also a promise: that the Malaysians will continue what Professor Rampal and his generation started. That we will not falter in the face of industry pressure, policy fatigue, or social apathy. That we will end this epidemic not with silence, but with science, solidarity, and strength.

### MOVING FROM TOBACCO CONTROL TO TOBACCO ENDGAME

Endgame generally refers to a goal to reduce smoking prevalence to less than 5% by a specific year. The strategies aim to rapidly and permanently reduce tobacco use to minimal levels, effectively ending the tobacco epidemic, which refers to the situation where the tobacco epidemic is to be ended rather than controlled. Malaysia has set a target to reduce the prevalence rate to less than 15% by 2025 and to less than 5% by 2040. We are now at a critical juncture. We have achieved substantial progress over the years, and more recently, with the passage of the Control of Smoking Products for Public Health 2024 (Act 852). For Malaysia to be "A Smoke Free and Healthy Nation," a paradigm shift is needed in developing regulatory reduction in demand-supply strategies to address addictive substances.

There is a need for a paradigm shift from a focus on cure and care in the presence of ill health to promoting and maintaining good health. There is a need for a whole-of-government and community approach. There is a need for action by the entire government, including ministers, Members of Parliament, and State Assemblies. The politicians, NGOs, health care providers, community leaders, mass media, and the population must cooperate with the Government. Their roles include advocacy, community leadership, educational, catalyst, capacity building and training, Research, and Complementary roles. They must understand that countering the tobacco industry's tactics and practices is crucial for the success of tobacco control policies. It is also essential to realise that tobacco products are the only legally available products that can kill up to one-half of their regular users if consumed as recommended by the manufacturer. The industry must also play its role as a responsible corporation and work towards eliminating this menace. In Malaysia, it is estimated that 829,000 students are enrolled in primary and secondary schools. This includes both government and private institutions. Most of these students, about 3,085,000, are enrolled in primary education, and over 1.2 million are pursuing tertiary education. There have been reports in the mass media that there are students

who smoke and vape. There must be a paradigm shift in the policies of the Ministry of Education; they must play their role and be more involved in this National problem. They must not only be aware but also understand and participate in the strategies and processes. The University staff in Malaysia, encompassing both academic and administrative personnel, must play a more initiative-taking role. The Ministry of Digital is committed to developing artificial intelligence (AI) talent and integrating technology into daily life. It plays a significant role in tackling the number one killer in Malaysia. The Ministry initiatives should include a collaboration with the MOH and community leaders to train participants in AI, providing a platform for talent development, and driving AI transformation across sectors. They must play a role in the collaboration of the MOH in countering the tobacco industry's exploitation of AI to target vulnerable populations and undermine policies. The Ministry of Local Government should play a more active role in enforcing the provisions under ACT 852. The Ministry of Finance's role should not be limited to developing economic policy but also lead to a healthy Nation. MMA has been recommending increasing taxes on tobacco products as a strategy for reducing smoking rates in Malaysia. It is widely regarded as a highly effective strategy for reducing tobacco use and its consequences. Higher prices make tobacco less affordable, leading to decreased consumption, particularly among young people and low-income populations. At the recommendation of MMA, the Government of Malaysia decided to increase the

tobacco tax and later chose to tax smoking, liquor, and gambling, and called it the Tax Haram (Sin tax). The increased tobacco taxes generate revenue amounting to billions of ringgits. Currently, instead of using this increased revenue to fund public health programs aimed at further reducing tobacco use, it has been channeled to the Government's Consolidated Fund. In this central account, all government revenues and receipts are deposited. The World Bank has recommended that the tax on tobacco and tobacco products be at least 70% of the retail price. At several meetings of the WHO FCTC Steering Committee at the MOH, in the presence of a representative from the Ministry of Finance, I have inquired why the tax has not been increased in the last few years. I have also asked why the money collected from the Tax Haram on Tobacco products is not used for prevention, for smokers who want to quit, and for the prevention of non-smokers from starting smoking. I have also inquired whether the Ministry of Finance has informed the public that the revenue from Sin Tax is channeled to the Government Consolidated Fund. In this central account, all revenues and receipts are deposited. The revenue generated from the syntax was to fund public health programs aim at reducing tobacco, alcohol, and gambling. The representative has, to this day, not given a satisfactory answer. Every Malaysian has a role to play in reaching the target set for the endgame. We all need to be more proactive.

# A single centre study on prevalence of anaemia in children and adolescents with atopic dermatitis and its associated factors

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## ABSTRACT

**Introduction:** Chronic inflammation, food avoidance, and the use of systemic immunosuppressants are associated with anemia in patients with atopic dermatitis (AD). This study aimed to determine the prevalence of anemia among children and adolescents with AD, the type of anemia, and its predisposing factors.

**Materials and Methods:** This is a cross-sectional study. Patients aged  $\leq 18$  years with AD were included in the study. The exclusion criteria were malignancy, chronic organ failure, and haematological diseases. Dietary patterns and nutrient intake were determined using a 3-day dietary recall. AD severity was assessed using a scoring tool known as Scoring Atopic Dermatitis (SCORAD). Peripheral blood counts, iron studies, and serum B12 and folate levels were determined.

**Results:** A total of 77 patients were recruited for this study. The mean age was  $8.58 \pm 5.26$  years with 43(55.8%) boys and 34(44.2%) girls. Most (58.4%) were from economic bottom-tier households with incomes below RM4850. Food avoidance was common (55.8%), primarily for shellfish (37.7%), nuts (33.8%), and eggs (22.1%). The prevalence of anemia was 58.4%, and 71.1% was due to iron deficiency. Younger age, male sex, underweight, and lower intakes of iron, protein, and fat were significantly associated with anemia. There was no association between the severity with anemia.

**Conclusion:** The high prevalence of iron deficiency anemia in patients with AD was likely due to the lower intake of food restrictions compounded with chronic inflammation in AD.

## KEYWORDS:

Anaemia, iron, atopic dermatitis, nutrition

## INTRODUCTION

Atopic dermatitis (AD) is an inflammatory skin disorder that impacts around 20% of children and 10% of adults.<sup>1</sup> Prevalence of AD in Malaysian children was 13.4% in 2018.<sup>2</sup> The pathophysiology of AD is a complex interplay between genetic, immune system, skin barrier and environmental

factors.<sup>1</sup> Nutrition is important for a healthy immune system. Micronutrients play a role in regulating immunity, improving the skin barrier function, and preventing itching in AD.<sup>3-6</sup>

Anemia is associated with many factors. The impact of anemia in children includes growth delays, weakened immune system, impaired motor and cognitive development, and reduced stamina.<sup>7</sup> In Malaysia, the reported overall prevalence of anemia was 21.3%.<sup>8</sup> A study conducted in Penang highlighted anemia as a public health issue among Malaysian children, with a prevalence of 22.3%.<sup>9</sup> A higher percentage (48.5%) of aboriginal school children aged seven to 12 years were diagnosed with anemia in a remote area of Pahang, Malaysia.<sup>10</sup> Studies conducted in Korea and the US indicated that children with atopic conditions have a significantly higher incidence of iron deficiency anemia<sup>12-13</sup>. The prevalence of anemia among paediatric population was 3.46% in South Korea.<sup>13</sup> In Turkey, the rate of iron deficiency anaemia was greater in patients with AD (15%) compared to those without (5%) AD.<sup>14</sup> Prevalence of anaemia among the paediatric population with AD was 4.3% in South Korea.<sup>13</sup>

Chronic inflammation, food avoidance, and the use of systemic immunosuppressants in atopic diseases are associated with anaemia.<sup>13-16</sup> Parents of children with AD often restrict certain foods, favoring fruits and vegetables over dairy, meat, and fish owing to concerns about allergies.<sup>5-6, 16</sup> Iron loss in AD-related malabsorption and pruritus due to iron-deficiency anemia can worsen skin lesions, creating a vicious cycle.<sup>17</sup>

This study aimed to determine the prevalence of anemia among children and adolescents with AD. The type of anemia and its predisposing factors, including socioeconomic factors, AD severity, and dietary restriction, were assessed.

## MATERIALS AND METHODS

### Study Design and Participants Selection

This was a single-center cross-sectional study. Patients with AD attending the dermatology clinic at Hospital Tuanku Jaafar Seremban, Negeri Sembilan, Malaysia were screened and recruited from May 1, 2023, to October 31, 2023. Patients

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aged  $\leq 18$  years who met the diagnostic criteria for AD based on the UK Working Party were included. The exclusion criteria were malignancy, chronic or end-stage organ failure, hemoglobinopathy, bleeding disorders, menorrhagia, rheumatological disorders, inflammatory bowel disease, severe bronchial asthma, and systemic drugs that can cause cytopenia.

### Data Collection

Demographic details, history of AD, comorbidities, and treatment history were obtained by interviewing patients or their caregivers and reviewing their medical records. Body mass index (BMI) was calculated and plotted using the World Health Organization (WHO) BMI for the age percentile chart. 18 Investigator Global Assessment (vIGA), ScoRing Atopic Dermatitis (SCORAD), and Dermatology Life Quality Index (DLQI) scores were calculated.<sup>19-21</sup> AD severity was categorized according to the SCORAD index as mild ( $<25$ ), moderate (25-50), and severe ( $>50$ ). 19 vIGA scores were used to categorize disease severity as mild (0-2), moderate (3), and severe (4). The DLQI scores were categorized as  $<10$  or  $\geq 10$ .<sup>20-21</sup>

Patients were asked to recall a 3-day dietary intake, which consisted of two weekdays and one weekend. This included food allergies, types of food that were avoided, types of food or beverage consumed, portion size, and cooking styles. Patients were interviewed regarding the food consumed over the past 24 hours, and portion sizes were determined using common guides such as cups, bowls, tablespoons, and teaspoons. The intake of key food groups was analyzed, including meat (chicken, beef, pork), seafood (fish, crustaceans, mollusks), vegetables, eggs, milk, peanuts, and snacks (cakes, sweets, and chips). Dietary intake was analyzed for macronutrients and micronutrients using Nutritionist Pro™ Software (Axxya Systems, the United States Department of Agriculture (USDA) Standard Reference Database, First DataBank, Inc., San Bruno, California). Malaysian food composition databases, as well as other international databases such as the USDA Food Database, Canadian Food Database, and Mexican Food Database, are included in this software.

Venous blood was obtained for peripheral blood count, iron study, and serum vitamin B12 and folate levels. Peripheral blood counts were analyzed using a Sysmex XN3000e. A Siemens Atellica Solution Analyzer was used for iron studies and measurements of serum B12 and folate levels. An iron study was performed using the colorimetric method, while folate and B12 studies were performed using a chemiluminescent paramagnetic microparticle immunoassay. These tests were conducted in the laboratory of the Hospital Tuanku Jaafar. Anemia was diagnosed based on the WHO definition: hemoglobin (Hb)  $<11$  g/L in children aged  $<5$  years, while for those aged  $\geq 5$  years, anemia was defined as  $<12$  g/L in females and  $<13$  g/L in males. The type of anemia was determined by a combination of hemoglobin value, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), iron, transferrin saturation, and B12 and folate levels. Comprehensive evaluations of hepcidin, erythropoietin, and transferrin levels, which may influence iron deficiency anemia, were not performed, as these are not required for the clinical diagnosis of anemia.

### Sample size determination

This study was approved by the Medical Research and Ethical Committee (MREC), Malaysia, under the research code NMRR-20-00719-WZO. The sample size was calculated using the population proportion formula based on the results reported by Rhew et al. on the association between atopic disease and anemia in children. Seventy-seven participants were required for the estimated 95% confidence level and 5% absolute precision.<sup>22</sup>

### Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 23. Descriptive statistics were used to analyze the sociodemographic and clinical characteristics of the patients. Categorical variables are expressed as frequencies and percentages. Continuous variables are expressed as means and standard deviations. Differences between categorical variables were compared using Pearson's chi-square or Fisher's exact tests. Differences between continuous variables were compared using the independent t-test or Mann-Whitney U test. Binary logistic regression was used to determine the predisposing factors for anemia. Statistical significance was set at  $P < 0.05$ .

## RESULTS

### Demographic Characteristics

A total of 77 patients participated in the study. The mean age was  $8.58 \pm 5.26$  years. There were 43 boys (55.8%) and 34 girls (44.2%). The majority of the patients were Malay (70.1%), followed by Chinese (19.5%), and Indian (10.4%). Most children (58.4%) were from economic bottom-tier households with income below RM4850. Nineteen (24.7%) patients had concomitant allergic rhinitis and 9 (11.7%) had bronchial asthma. Table I.

### Clinical Characteristics and Laboratory Parameters

The mean BMI of the patients was  $17.59$  kg/m<sup>2</sup>. The majority of the study population had mild-to-moderate AD, with 53 (68.8%) categorized as mild, 19 (24.7%) as moderate, and 5 (6.5%) as severe. Most patients (74%) reported minimal impact on their quality of life, with a DLQI score of less than 10. All patients received topical treatment, 9.1% underwent phototherapy, and none of the patients received systemic therapy.

The mean hemoglobin level was  $12.16 \pm 1.51$  g/dL. The mean levels of MCV, MCH, iron, and transferrin saturation were below the normal range, whereas both B12 and folate levels were normal. The prevalence of anemia in our study population was 58.4%, and 71.1% was due to iron deficiency. One patient had iron and folate deficiencies. The majority (86.6%) of the patients with anemia had hemoglobin levels of more than 10 g/dl, only 7.8% had hemoglobin levels of 9-10 g/dl. Twenty-four (31.2%) patients had high eosinophil counts. A detailed analysis of the laboratory parameters is presented in Table II.

### Dietary History

Nearly half of the parents (46.7%) thought that their child had food allergies, and only one patient underwent an IgE test. Food restriction was common, and 43 (55.8%)

Table I: Characteristics of the study population

Characteristics	N= 77 n (%)
Age, mean±SD	8.58±5.26
Gender	
Male	43 (55.8)
Female	34 (44.2)
Ethnicity	
Malay	54 (70.1)
Chinese	15 (19.5)
Indian	8 (10.4)
Education level	
Underage for school	16 (20.8)
Primary	38 (49.4)
Secondary	21 (27.3)
Tertiary	2 (2.6)
Household income	
B40	45 (58.4)
M40	28 (36.4)
T20	4 (5.2)
Smoking status	
No	77 (100.0)
Alcohol use	
No	77 (100.0)
Other atopic diseases	
Allergy Rhinitis	19 (24.7)
Allergy Conjunctivitis	0 (0)
Bronchial asthma	9 (11.7)

participants actively avoided certain types of food. The most common restricted foods were shellfish (37.7%), nuts (33.8%), and eggs (22.1%), whereas 10% avoided dairy products.

#### Factors Associated with Anemia

Age and gender were significantly associated with anemia, and younger patients were more likely to have anemia. The prevalence of anemia was higher in males than in females ( $p=0.006$ ). Ethnicity, household income, concomitant allergic rhinitis or bronchial asthma, food restriction, and AD severity were not significantly associated with anemia (Table IV). Anemia was associated with lower protein, fat, iron, and folate intakes (Table V). There was high intake of carbohydrates, fat, and protein. We observed that some patients over-reported their food intake and parents tended to repeat the same main meals during the recall period.

Multiple logistic regression analyses did not reveal a significant independent risk factor for anemia other than the male gender (adjusted OR 0.27, 95%CI 0.10 - 0.70,  $p < 0.01$ ).

#### DISCUSSION

More than half of our study population had anemia and almost all were due to iron deficiency. The micronutrient status affects the risk of AD by altering cell-mediated immunity.<sup>17,24</sup> Iron is an important micronutrient involved in the regulation of cytokine production, cell growth, cell differentiation, and cell proliferation. The immune response in AD is dominated by type 2 cytokines including IL 4,5 and 13 which impairs the skin barrier, reduce antimicrobial peptide activity, and promote inflammation.<sup>24</sup> Iron modulates the differentiation and proliferation of Th cells, particularly Th1, Th2, Th17, and Treg cells.<sup>25</sup> Iron deficiency

favors a Th2 response as DNA synthesis in Th1 but not Th 2 is suppressed.<sup>17,24</sup> In addition, macrophages and neutrophils exhibit reduced bactericidal activity, while NK-cell's differentiation and proliferation are inhibited.<sup>17</sup> Adequate levels of iron enhance immune resilience and promote immune tolerance.

The prevalence of anaemia in patients with AD has been documented in previous reports.<sup>12-14,26</sup> The prevalence of iron deficiency anaemia (IDA) among children with AD in Turkey was 15%,<sup>14</sup> while in Korea, the prevalence was lower at 4.3%.<sup>13</sup> The prevalence of IDA or anemia due to inflammation was 57%.<sup>26</sup> The prevalence of anaemia among those with eczema were up to 3.0% in the 1997-2013 US National Health Interview Survey (NHIS) among 207 007 children and adolescents.<sup>12</sup> The adjusted odds ratio for anaemia in this survey was 1.83; 95% CI, 1.58-2.13.<sup>12</sup> A cross sectional study which analysed data from 1468 033 patients in the 2016 National Health Insurance Service data set found significant associations between iron deficiency anaemia and atopic conditions.<sup>26</sup> The odds ratios (OR) for IDA in individuals with atopic dermatitis, allergic rhinitis, and asthma were 1.40 (95% CI, 1.33-1.48;  $p < 0.001$ ), 1.17 (95% CI, 1.14-1.21;  $p < 0.001$ ), and 1.32 (95% CI, 1.28-1.36;  $p < 0.001$ ), respectively.<sup>26</sup> This study also documented a higher prevalence of anaemia with higher number of atopic diseases, suggesting that the inflammatory state of atopic diseases contributes to increased risk of anemia.<sup>26</sup> The prevalence of anaemia in our study population was very high compared to data from other countries. However, anemia was mild in most of the patients. We postulate that the higher prevalence is due to the practice of food restriction in addition to the effect of chronic inflammation in AD. A previous local study showed that up to 61% of AD patients

Table II: Clinical characteristics, treatment and laboratory parameters

Clinical characteristics	N=77 N (%) or mean $\pm$ SD
Weight, kg	29.91 $\pm$ 18.24
Height, cm	124.08 $\pm$ 28.80
BMI, kg/m <sup>2</sup>	17.59 $\pm$ 5.31
AD severity, SCORAD score	
Mild (< 25)	53 (68.8)
Moderate (25 – 50)	19 (24.7)
Severe (> 50)	5 (6.5)
Quality of life, DLQI score	
<10	57 (74.0)
> 10	20 (26.0)
Treatment for AD	
Topical	77 (100.0)
Phototherapy	7 (9.1)
Systemic therapy	0 (0)
Laboratory parameters (normal range)	
Haemoglobin, g/dL	12.16 $\pm$ 1.51
Age < 5 years (11-15.5g/dL)	
Age $\geq$ 5 years	
Females (12-15.5g/dL)	
Males (13-15.5g/dL)	
Eosinophil, 103/ $\mu$ L (0.10-1.00 103/ $\mu$ L)	12.16 $\pm$ 1.51
Iron, $\mu$ mol/L ( 11.6-31.3 $\mu$ mol/L )	0.83 $\pm$ 0.75
TIBC, $\mu$ mol/L ( 44.75-76.08 $\mu$ mol/L )	11.42 $\pm$ 5.56
Ferritin, ng/ml ( 22-322 ng/mL )	30.76 $\pm$ 23.18
MCV, fL (77.0-95.0 fL)	75.89 $\pm$ 7.21
MCH, pg (25.0-33.0 pg)	24.43 $\pm$ 3.00
B12, pmol/L (156-672 pmol/L)	444.65 $\pm$ 209.19
Folate level, nmol/L (> 12.19 nmol/L)	25.65 $\pm$ 12.78
TSAT, %	18.17 $\pm$ 8.69
Eosinophil count > 1 103/ $\mu$ L	24 $\pm$ 31.2
Anaemia status	
No	32 (41.6)
Yes	45 (58.4)
Iron deficiency, n=45	
No	13 (28.9)
Yes	32 (71.1)
B12 deficiency, n=5	
No	45 (100)
Yes	0 (0)
Folate deficiency, n=45	
No	44 (0.98)
Yes	1 (0.02)

avoided eating three or more food groups.<sup>16</sup> Health practitioners caring for AD patients should be aware of the risk of not just anemia but other nutritional deficiencies as well as institute appropriate measures to diagnose and manage these conditions.

The etiology of anemia in patients with AD includes anemia of chronic disease and nutritional deficiencies (iron, vitamin B12, and folate) due to a restrictive diet and side effects of medications such as azathioprine, methotrexate, and Janus kinase inhibitors. There is limited research exploring the link between eczema and anemia. The use of systemic immunosuppressants, malnutrition, and use of alternative medicine increased the risk of anemia.<sup>13</sup> IDA was linked to early onset AD (< 2 years of age), history of atopy, skin infections, and comorbid allergic conditions, especially bronchial asthma.<sup>14</sup> IDA prevalence was 26.7% in individuals with one atopic disease and 53.3% in those with multiple atopic diseases, indicating a higher risk with more atopy.<sup>14</sup> the Prevalence of anemia was higher in males and younger

children in a nationwide cross-sectional survey that determined the status of malaria and anemia in children between the ages of 6 months to 14 years.<sup>25</sup> Household income was not associated with anemia among our study population, which contradicts results from previous research.<sup>26-28</sup> This discrepancy may be due to the fact that most of our study participants and patients at our center were from the lower-income group.

Anemia among our patients was not associated with AD disease severity, which contrasted with the results of a study investigating risk factors for anemia in children with AD in Turkey; however.<sup>14</sup> Our study population did not show this association, likely because the majority of our patients had mild AD. Chronic inflammation in AD has been shown to hinder iron absorption and utilization even if dietary intake is adequate.<sup>12</sup> This explains why patients with AD are at an increased risk of anemia, even in the absence of nutrient deficiencies.

Table III: Dietary pattern of the study population

Dietary history	N=77	N (%)
Patient's self-diagnosed food allergy	36 (46.7)	
Types of patient-reported food causing allergy		
Shellfish	20 (54.1)	
Nut	1 (2.7)	
Chicken	3 (8.1)	
Brinjal	1 (2.7)	
Egg	11 (29.7)	
Yeast	1 (2.7)	
Vegetarian	4(5.2)	
Food avoidance	43 (55.8)	
Types of Food Avoided		
Shellfish	29 (37.7)	
Nuts	26 (33.8)	
Egg	17 (22.1)	
Dairy	8 (10.4)	
Cow milk	6 (7.8)	
Soy	7 (9.1)	
Beef	5 (6.5)	
Wheat	5 (6.5)	
Poultry	5 (8.5)	
Fish	5 (6.5)	
Cakes & sweets	3 (3.9)	
Fruits	0 (0)	
Vegetables	0 (0)	
Others types of food	17 (22.1)	

Food restrictions were common among our patients. This finding was similar to a report from Korea, where the prevalence of food restriction ranged from 48.1% to 50.0%.<sup>6</sup> A notable proportion of parents (55%) of children with AD believed their child had food allergies compared to 12% of parents of children without AD.<sup>29</sup> Food allergy were self-reported but not verified through clinical confirmation in our study. Thus, it is possible that many cases were not true allergies. Shellfish was the most restricted food in this study and previous studies conducted in Korea and Malaysia.<sup>6,16</sup> Other frequently avoided foods included milk, dairy products, eggs, and nuts.

Iron deficiency may occur in patients with AD who follow restrictive diets to avoid triggering a flare, which subsequently leads to insufficient iron intake. Our study showed that patients with anemia had a significantly lower intake of iron, protein, and fat. Thus, low iron intake is a contributing factor to iron deficiency anemia. Anemia was also associated with younger age and lower BMI; however, the only significant independent risk factor was male sex. Folate and B12 levels were slightly lower than the recommended nutrient (RNI) for Malaysia, but this was not a contributing factor to anemia in this study, as both B12 and folate levels were within the normal range and the intake for patients with and without anemia were similar. The calorie intake was adequate, whereas the intake of carbohydrate, protein, and fat was higher than the recommended nutrient intake (RNI) for Malaysia. This could be attributed to multiple factors. The validity of dietary recall depends on the accuracy of respondents' recall of their food consumption. Poor recall can lead to repetition of meals and overreporting.<sup>30</sup> Food insecurity, defined as limited access to safe and nutritious food, is influenced by socioeconomic,

demographic, and environmental factors. Children in food-insecure households often consume energy-dense, nutrient-poor foods that leads to poor diet quality.<sup>31-32</sup> Studies have suggested that low income and financial constraints often lead to meal repetition, as families choose inexpensive and easy-to-prepare foods.<sup>33</sup> The convenience of meal preparation, especially for busy families often results in repetitive meals particularly when affordable, processed foods are readily available.<sup>34</sup> Many parents resorting to food exclusion diets without guidance from healthcare providers may also lead to energy dense diets.<sup>29</sup>

Our study is limited by the possibility of recall bias in three-day dietary recall. Future studies should consider prospective food records, 24-hour recall, or food frequency questionnaires to reduce the possibility of recall bias. However, the serum levels of micronutrients that are important for anemia were objectively measured. Concurrent parasitic infections were not assessed in this study. To further validate our findings and address the limitations of this study, a multicenter study with a larger sample size would be valuable, allowing for more comprehensive analysis and greater generalizability of the results.

## CONCLUSION

The prevalence of iron-deficiency anemia is high in patients with AD. Food restriction was common with lower intakes of protein, fat, iron, and folate among AD patients with anemia. Younger age, male sex, and underweight were factors associated with anemia. In summary, food restriction leading to inadequate iron intake may be a key factor in iron deficiency anemia among our group of patients. Chronic inflammation may also play a role in atopic eczema.

**Table IV: Factors associated with anaemia**

Parameters	Anaemia, N=77 n (%)		Statistical test	p value
	No N= 32	Yes N= 45		
Age, mean ± SD	10.63 ±4.26	7.13±5.46	3.02 (75.0) <sup>a</sup>	0.003*
Gender			7.47 (1) <sup>b</sup>	0.006*
Male	12 (37.5)	31 (68.9)		
Female	20 (62.5)	14 (31.1)		
Ethnicity			-	0.327 <sup>c</sup>
Malay	20 (62.5)	34 (75.6)		
Chinese	7 (21.9)	8 (17.8)		
Indian	5 (15.6)	3 (6.7)		
Education level			-	0.002 <sup>c*</sup>
Underage for school	1 (3.1)	15 (33.3)		
Primary	19 (59.4)	19 (42.2)		
Secondary	10 (31.3)	11 (24.4)		
Tertiary	2 (6.3)	0 (0.0)		
Household income			-	0.534 <sup>c</sup>
B40	17 (53.1)	28 (62.2)		
M40	14 (43.8)	14 (31.1)		
T20	1 (3.1)	3 (6.7)		
Food restriction			0.76 (1) <sup>a</sup>	0.384
No	16 (50.0)	18 (40.0)		
Yes	16 (50.0)	27 (60.0)		
BMI category			-	0.011 <sup>c*</sup>
Underweight	16 (50.0)	34 (75.6)		
Normal	14 (43.8)	7 (15.6)		
Overweight	1 (3.1)	4 (8.9)		
Obese	1 (3.1)	4 (8.9)		
Extreme obesity	1 (3.1)	0 (0.0)		
AD severity			1.02 (1) <sup>b</sup>	0.312
Mild	20 (62.5)	33 (73.3)		
Moderate to severe	12 (37.5)	12(26.6)		
Eosinophil			0.00 (1) <sup>b</sup>	0.990
Normal	22 (68.8)	31 (68.9)		
High	10 (31.3)	14 (31.1)		
Concomitant allergic rhinitis			0.23 (1) <sup>b</sup>	0.631
No	25 (78.1)	33 (73.3)		
Yes	7 (21.9)	12 (26.7)		
Bronchial asthma			-	0.152 <sup>c</sup>
No	26 (81.3)	42 (93.3)		
Yes	6 (18.8)	3 (6.7)		

<sup>a</sup>Independent t-test; <sup>b</sup>Pearson Chi square; <sup>c</sup>Fisher's exact test; <sup>d</sup>Mann Whitney test; \* Statistically significant

**Table V: Nutrient intake ( % RNI ) among patients with and without anemia**

Dietary intake,	Anemia N=77 Median (IQR)		P value
	No N= 32	Yes N= 45	
Calorie, %	94.68 (53.10)	76.85 (28.16)	0.059 <sup>d</sup>
Protein, %	291.66 (202.03)	211.10 (158.80)	0.036 <sup>d*</sup>
Carbohydrate, %	170.19 (49.82)	159.44 (31.62)	0.251
Fat, %	8460.00 (6342.00)	7375.00 (2851.00)	0.009 <sup>d*</sup>
Iron, %	138.32 (70.37)	79.94 (63.42)	0.016*
B12, %	58.50 (147.84)	40.26 (81.57)	0.079 <sup>d</sup>
Folate, %	22.53 (24.98)	27.60 (49.12)	0.009 <sup>d*</sup>
Vitamin A, %	183.08 (131.53)	210.27 (149.74)	0.189 <sup>d</sup>
Vitamin C, %	105.43 (131.81)	54.35 (111.64)	0.077 <sup>d</sup>

<sup>a</sup>Independent t-test; <sup>b</sup>Pearson's chi-squared test; <sup>c</sup>Fisher's exact test; <sup>d</sup>Mann Whitney test; \*statistically significant.

## ACKNOWLEDGEMENTS

We would like to thank Persatuan Dermatology Malaysia for the research grant. The funding organization had no role in the study design, data collection, analysis, interpretation, or preparation of this manuscript. We would also like to thank the Director of the General of Health Malaysia for their permission to publish this article.

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# Learning from the plain film competency assessment in the Singapore radiology residency programme

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## ABSTRACT

**Introduction:** The national Plain Film Competency Assessment (PFCA) was held to allow passing Residents to report plain radiographs independently and thus perform on-call night duties. Our study aimed to investigate factors influencing performance in this exam.

**Methods:** This was a retrospective study of data from 2012-20. Training data included previous experience in radiology as well as number of reported films (total and by body regions) whilst examination data comprised these scores as well as error analysis (classified as False Positives, False Negatives or Incorrect Abnormality) for the main and repeat sittings.

**Results:** Up to 222 residents were included for statistical analysis. Those with previous radiology exposure were more likely to pass, 85.1% compared to 68.9% ( $c=0.03$ ) and had higher scores, 85.1 versus 81.3 ( $p<0.01$ ). The number of co-reported films did not affect passing even after excluding those with previous experience. Chest and limb radiographs made up at least three-quarters of the reported films, with residents passing these whilst failing those from the abdominal, skull and spine regions. There was no significant difference between their improved scores after undergoing remediation of either one or four months in duration. However, error analysis after remediation revealed a lower combined percentage of False Positives and Negatives.

**Conclusions:** Experience in a radiology posting after graduation facilitated passing. Although we could not establish an association between the number of reported films and passing, there was an imbalance in the variety of reported films, which should first be resolved.

## KEYWORDS:

*Diagnostic radiology, plain film training and assessment, residency programme, fellow of the Royal College of Radiology (FRCR) examination*

## INTRODUCTION

Postgraduate specialist medical training in Singapore adopted the American-style Accreditation Council for Graduate Medical Education International (ACGME-I) system in 2010.<sup>1</sup> After graduating from medical school and

completing the compulsory one-year internship, doctors can continue rotating through various medical disciplines as a Medical Office Posting Exercise Medical Officer (MOPEX-MO), which is a non-trainee position, or begin specialist training by joining the Residency Programme (RP). In radiology RP, local hospitals are divided into three clusters (Central, Western and Eastern). The training program in each cluster is helmed by a Programme Director (PD) based in the Sponsoring Institution with Residents rotating in-and-out of that hospital to Participating Sites (other hospitals within the same cluster) and occasionally across clusters.

Amidst the availability of higher-order imaging modalities and image-guided interventions expanding in tandem with artificial intelligence, the interpretation of plain radiographs remains a fundamental tenet of radiology.<sup>2,3</sup> Therefore, Residents spend a large portion of the initial year of their 5-year programme learning to interpret them. Prior to the RP, traineeship resembled an apprenticeship, where feedback from “buddy” reporting and/or in-house assessments determined whether a trainee could report independently and perform on-call night duties.<sup>1,4</sup> These had a degree of subjectivity and were conducted within the respective institutions instead of at the national level, making them an improper gauge of a trainee’s preparedness for this task. Unfortunately, this method continues to be applicable to the radiology MOPEX-MOs. With the RP, ACGME-I mandated that every film be co-reported with a specialist and signed out by the latter, but allowance was given for Singapore to hold a national Plain Film Competency Assessment (PFCA) midway through their first year of residency training (RY1) to allow passing residents to report independently and perform night calls. Despite being granted this latitude, the passing residents are continually audited to ensure patient safety.<sup>5</sup>

There have been opposing views regarding mastering image-interpretation exams, such as the PFCA, with some believing it to be number-based and others contending it to be time-dependent.<sup>4,6</sup> Our prerequisite of having co-read a minimum of 600 films to be eligible to sit for the PFCA had been an arbitrary one, and if the volume was an issue, this number would need to be tweaked. The remediation period had also been raised from one to four months, starting from the fourth year of conducting this exam, with the unvalidated assumption that a longer duration would serve this purpose better. Hence, the objectives of our study to investigate (1) the

*This article was accepted: 12 May 2025*

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number and variety of films, (2) the period of remediation, and (3) other experiential factors that may influence performance in the PFCA comes at a timely juncture as it has been almost a decade since its inception. Given the enduring British influence in training, our residents also sit for the Fellow of the Royal College of Radiology (FRCR) Part 2B (final) Examination as early as RY3.<sup>1</sup> We hope that our findings will benefit PDs and their residents during plain film training and in preparing for both the PFCA as well as the FRCR 2B, which the former was modelled after.

## MATERIALS AND METHODS

### Study design

PFCA parallels the rapid reporting component of FRCR 2B albeit with simpler parameters.<sup>5</sup> First, it comprised two papers (Papers A and B) with 30 questions each instead of one paper in the FRCR, with the pass mark arbitrarily set at an average of 80% compared to 90% for the latter.<sup>5,7-9</sup> We envisaged that a single paper of 30 questions might not be representative, if biased by exam anxiety or unfamiliarity with the equipment, this being their first major hurdle after beginning residency.<sup>10,11</sup> Instead of having a solitary 60-question paper that would have been too strenuous, we simply doubled the number of papers to maintain the format. Second, each paper in the PFCA was allotted 60 min (with a half-hour interval in between) in contrast to 35 min in the FRCR.<sup>7,11</sup> Each paper consisted of a mixture of normal and abnormal films (40-60% of each), chosen to replicate those typically encountered during daily work.<sup>7</sup> If an abnormality was identified, the candidate had to fully describe the pathology. The PFCA was administered and marked by a board of examiners selected from different training hospitals across Singapore as well as by expertise in those body regions. The main sitting (1st attempt) was held around the 7th month after commencing training. For unsuccessful candidates, there was one resit (between 2012-14) in the 8th month, or two resits (from 2015-20) in the 11th (2nd attempt) and 12th (3rd attempt) months. After the initial few runs, the committee was able to adjust several variables which then formed a blueprint for subsequent sittings. One of these was stratifying abnormal films into easy, moderate and difficult grades, with approximately half of them being easy. Another was having an almost equal proportion of truncal (i.e. chest, abdominal, skull and spine) as well as appendicular (i.e. limb) radiographs, reflecting those from general practice and the emergency department. The last was having a longer period of remediation with the belief that it may fulfil its intended purpose and yield better results.

Error analysis was only provided for failing candidates to guide remediation. These were classified into "False Positive" (i.e. identifying a normal film as abnormal), "False Negative" (i.e. missing to observe an abnormality) and "Incorrect Abnormality."<sup>5</sup> Whilst the first two were more perceptual in nature, the last group dealt with cognitive errors.<sup>12</sup> It usually includes mixing-up the sides, erroneous spelling (e.g., coronoid versus coracoid), or incorrect nomenclature (e.g., metatarsal instead of metacarpal) but occasionally reflects insufficient experience and hence attributing abnormality to the inappropriate structure (e.g., mediastinal instead of pulmonary mass) or inability to conclude accurately (e.g.,

diagnosing malignancy rather than benignity). This was a local initiative afforded to candidates to guide them in their formative years with the mode of remediation left to the respective PDs.

### Study population and eligibility criteria

First year residents would undergo an initial six months of supervised reporting. They co-reported these films with senior staff, with the latter signing out the final report until they passed the PFCA. Residents were required to keep a log of the number and types of plain radiographs that they had co-read, and a minimum of 600 films were required before they were eligible to sit for this exam.<sup>5</sup>

### Statistical analysis

We conducted a retrospective study using data from 2012 to 2020. All resident data collated by the respective PDs and the examination data collected by the PFCA examination committee were reviewed. Data from the PDs included previous experience in radiology (categorized based on intensity of exposure) as well as the number of films (total and proportion by body regions) co-reported since beginning residency training. Given the retrospective nature of this study, our figures were retrieved from resident logbooks as well as Patient Archiving and Communication Systems (PACS). The examination data included scores and sub-scores for the main and repeat sittings. All data were anonymized and tabulated in password-protected Microsoft Excel 2018. Pearson's and intra-class correlation coefficients were calculated for the pair of papers of the main sitting, but not for the resits that had fewer candidates. Associations between categorical variables were tested using Pearson's chi-squared test, or Fisher's exact test if at least one of the expected cell counts was < five. Independent and paired t-tests were employed when there were two categories of continuous variables, while One-way Analysis of Variance (ANOVA) was employed when there were more than two of these, against nominal-level variables. All Statistical analyses were performed using IBM SPSS Statistics (version 29.0; IBM Corp., Armonk, NY, USA). Statistical significance was declared if the p-value was less than 0.05. This study was approved by the National Healthcare Group Domain Specific Review Board (reference no. 2020/01225).

### Outcome measures

When considering previous experience, the PDs graded the RP applicants according to whether they had performed an elective in radiology as an undergraduate or had worked as a radiology MOPEX-MO before joining the RP. There was even a handful who had come from abroad seeking employment in Singapore, and they had to enrol in the RP despite already passing the FRCR and accredited as a specialist in their home country. This was because their training program may not have been recognized by the Singapore Accreditation Board. Groups without and with previous experience were compared with respect to their ability to pass on their 1st attempt and their obtained scores to determine if this made a difference.

In order to determine the effect of number and variety of co-reported films, passing and failing residents on the 1st attempt were compared with respect to the number of radiographs they reported in total and by body regions, with

corresponding scores for the latter. We postulated that passing residents were likely to have reported more radiographs and performed better for each body region.

To study the effect of a shorter versus longer remediation period, the number who passed the resit and their scores were compared. The same was performed with regards to their error analysis subsequently. We hypothesized that a longer period of remediation would improve these outcomes.

Given that there were a pair of papers in each attempt, it would be beneficial to study the effect one paper had on the other. We hypothesized that residents would perform less consistently on the 1st sitting resulting in a lower correlation between pairs of papers and larger difference in these scores. For residents who passed one paper, the likelihood ratio of them passing the PFCA if it was Paper A was calculated for each sitting. We envisaged that the effect of anxiety on performance would be more profound on the initial rather than subsequent sitting.

Error analysis was performed for all candidates for the purpose of this study. The shift from False Positive or Negative towards Incorrect Abnormality may suggest acquisition of radiological proficiency with concomitant reduction of perceptual errors, and hence a lower tendency to over- or under-call an abnormality.

## RESULTS

There was a total of 222 Residents across this period, averaging 20-32 Residents per year. They were divided into the following three clusters: 61 (from the Central Cluster), 54 (from the Western Cluster) and 107 (from the Eastern Cluster). Review of the examination data revealed that 165 passed in one attempt, 46 in two attempts and 8 after three attempts. The remaining three either did not pass after exceeding the maximum number of tries or had dropped out before then.

### Results of the 1st attempt

The average scores for the Central, Western and Eastern Clusters were 83.5 (SD=7.7), 82.2 (SD=6.6), and 82.2 (SD=7.5), respectively, which were not statistically significant ( $p=0.496$ ). The 165 residents who passed scored 86.0 (SD=4.3), while the 57 who failed scored 72.6 (SD=4.7); the difference in their scores was significant ( $p<0.01$ ).

### Did previous rotation through radiology influence passing?

We divided previous exposure in radiology into four categories in order of increasing intensity

- Level 0 (n=138) – no previous experience in radiology
- Level 1 (n=10) – undertook a radiology elective during their final year of medical school. Such attachments were usually up to one month in duration. The experience gained was very general, and although they may be taught the basic principles of reading plain radiographs, they would not have co-reported any of these.
- Level 2 (n=68) – previous rotation through radiology as a MOPEX-MO
- Level 3 (n=6) – post-graduate specialist from overseas with FRCR

As the first two levels excluded radiology reporting, it was sensible to combine these. We found that 68.9% of those in Levels 0 and 1 passed at their 1st attempt compared to 83.8% of those from Level 2 and 100% of those from Level 3 which was significant ( $c=0.041$ ). Their respective scores were 81.3 (SD=7.3), 84.8 (SD=6.9) and 89.2 (SD=4.3), which was also statistically significant ( $p<0.01$ ).

### Effect of number and type of co-reported films

Missing logbook data involving seven Residents (across all clusters) as well as incomplete data of 19 Residents from the Western Cluster due to a cluster PACS upgrade availed us with 196 Residents for the subsequent calculations.

They co-reported an average of 1539.4 (SD=706.1) films before their 1st attempt. The 146 (72.4%) residents who passed co-reported an average of 1567.8 (SD=725.7) films. The 50 residents who did not pass co-read an average of 1456.4 (SD=644.9) films. This difference was not statistically significant ( $p=0.337$ ). As residents with previous radiology exposure (Levels 2 and 3) would have had a higher number of films unaccounted for, 127 residents from Levels 0 and 1 were considered separately. The 87 who passed the co-reported 1642.7 (SD=800.4) films compared to the remaining 40 who co-read 1471.0 (SD=681.4) films, but the difference was not statistically significant ( $p=0.242$ ). Chest and limb radiographs made up at least three-quarters of the reported films (Table I). On the average, residents had passing sub-scores for these body regions but failing sub-scores for the abdominal, skull and spine films. More specifically, unsuccessful residents had failing sub-scores in almost all body regions.

### Period of remediation

Fifty-six candidates underwent remediation (one had dropped out after the 1st attempt). Twenty Residents took the resit within a month and 36 after four months. With the shorter remediation, 90.0% passed whilst with a longer remediation 77.8% passed, with no significant difference in the pass rate ( $c=0.318$ ). The former showed an increase in scores from 71.9 to 83.2 ( $p<0.001$ ) and the latter, an increase in scores from 73.0 to 83.0 ( $p<0.001$ ). Despite the better scores after remediation, the improvement between these two groups was also not significant ( $p=0.920$ ).

### Comparison between the pair of papers in each attempt

The Pearson and intraclass correlation coefficients for the two papers of the main sittings were 0.337 and 0.495, respectively. There were 65 Residents who passed only one paper on their 1st attempt. The likelihood ratio of passing the 1st attempt if they passed Paper A was 1.87. This likelihood was reduced to 0.83 in their 2nd attempt. The difference in scores between the pair of papers in each attempt between those who passed and those who failed was not statistically significant, albeit with a narrower interval for the former on both occasions (Table II).

### Error analysis

In the 1st attempt, those who failed had a relatively higher percentage of False Positives and Negatives but lower Incorrect Abnormalities, all of which were not significant (Table III). Repeaters, regardless of whether they passed or failed, showed an increase in Incorrect Abnormalities to

**Table I: Distribution of reported radiographs by body regions as a proportion (%) of total films reported, and their corresponding sub-scores, stratified according to Residents who failed or passed the 1st attempt of the PFCA**

	Central Cluster		Western Cluster		Eastern Cluster <sup>a</sup>		Average
	Fail	Pass	Fail	Pass	Fail	Pass	
No. of Residents	18	41	10	40	21	75	205
%Chest (SD)	45.2 (9.6)	43.6 (11.6)	33.9 (11.7)	36.4 (7.3)	49.9 (8.1)	50.7 (7.3)	45.1 (10.9)
Sub-score (SD)	67.4 (14.1)	86.7 (11.5)	70.7 (17.6)	81.4 (13.3)	74.3 (12.7)	85.3 (11.0)	81.4 (13.7)
%Limbs (SD)	36.2 (9.5)	37.3 (10.2)	45.7 (12.4)	42.9 (9.3)	25.4 (8.1)	26.8 (7.3)	33.6 (11.3)
Sub-score (SD)	79.7 (7.7)	91.0 (6.3)	80.2 (6.1)	88.6 (5.8)	77.3 (6.6)	90.6 (5.7)	87.5 (8.0)
%Abdomen (SD)	11.5 (2.4)	11.9 (3.7)	10.1 (2.9)	9.7 (2.5)	15.8 (6.9)	13.6 (4.0)	12.3 (4.3)
Sub-score (SD)	70.7 (18.4)	88.9 (10.5)	59.0 (12.8)	79.1 (13.9)	67.8 (13.7)	81.5 (14.4)	79.1 (15.8)
%Skull (SD)	0.9 (0.7)	1.0 (0.6)	0.5 (0.6)	0.6 (0.6)	1.9 (1.0)	1.7 (1.0)	1.2 (1.0)
Sub-score (SD)	64.4 (22.8)	73.4 (21.8)	80.8 (16.7)	81.5 (24.6)	57.9 (26.0)	78.4 (23.7)	74.9 (24.1)
%Spine (SD)	6.3 (1.7)	6.3 (2.3)	9.9 (2.9)	10.5 (2.5)	7.1 (1.6)	7.2 (1.6)	7.7 (2.8)
Sub-score (SD)	63.6 (19.2)	76.3 (15.6)	70.7 (15.7)	81.6 (12.2)	64.0 (13.8)	75.3 (10.8)	74.3 (14.5)

<sup>a</sup>Ten Residents were excluded due to incomplete data by body regions

**Table II: Difference in scores between the pair of papers between those who passed versus those who failed in each attempt**

	Passed Mean (SD)	Failed Mean (SD)	Significance
1st attempt – difference in scores	7.5 (5.4)	8.1 (6.4)	0.481
2nd attempt – difference in scores	6.4 (5.0)	9.7 (7.3)	0.099

**Table III: Comparison of error analysis between those who passed versus those who failed in each attempt**

	Passed Mean (SD)	Failed Mean (SD)	Significance
1st attempt			
False positive (%)	24.83 (15.8)	28.38 (12.2)	0.128
False negative (%)	31.71 (16.4)	33.33 (14.6)	0.516
Incorrect abnormality (%)	43.46 (18.3)	38.29 (16.5)	0.064
2nd attempt			
False positive (%)	25.88 (17.8)	24.37 (8.8)	0.797
False negative (%)	26.38 (15.2)	26.57 (8.5)	0.485
Incorrect abnormality (%)	47.74 (19.4)	49.06 (10.6)	0.419

**Table IV: Error analysis for repeat candidates in each attempt as well as after stratifying them according to their duration of remediation**

	1st attempt Mean (SD)	2nd attempt Mean (SD)	Significance
False positive (%)	27.16 (12.1)	25.58 (16.4)	0.605
False negative (%)	30.51 (12.4)	26.42 (14.0)	0.142
Incorrect abnormality (%)	42.33 (11.7)	48.00 (17.9)	0.048
Short remediation (n=15) <sup>a</sup>			
False positive (%)	32.69 (10.5)	24.87 (17.0)	0.186
False negative (%)	24.16 (9.8)	26.29 (13.1)	0.655
Incorrect abnormality (%)	43.15 (12.1)	48.84 (14.9)	0.266
Long remediation (n=36)			
False positive (%)	24.85 (12.2)	25.87 (16.3)	0.774
False negative (%)	33.16 (12.5)	26.48 (14.6)	0.051
Incorrect abnormality (%)	41.99 (11.6)	47.65 (19.2)	0.108

<sup>a</sup>Five Residents were excluded due to incomplete data

almost 50% on the 2nd attempt. A paired-sample t-test revealed that this increase was significant overall (Table IV). Both short and long remediations confirmed these increases, but not significantly.

## DISCUSSION

Radiology teaching in medical schools is usually transmitted via lectures or opportunistic visits to the radiology department, while those for non-trainees are usually conducted by non-radiology personnel.<sup>13</sup> Unfortunately, nearly two-thirds of our residents enter RP under such circumstances. We found a statistically significant difference in passing the PFCA on their 1st attempt, and with higher scores, by residents who had had radiology experience after graduating compared to those without. This affirms our current practice of encouraging those intending to join radiology to rotate through as a MOPEX-MO beforehand, rather than dive straight into the RP. In addition to gaining insight into this specialty before committing, it is believed that this exposure facilitates swifter comprehension of radiology concepts upon commencing training.<sup>5</sup> An understanding of the sigmoid learning curve may explain why time spent as a radiology MOPEX-MO is not futile.<sup>14</sup>

We did not find a significant difference between the number of films read by those who passed or failed, even when those with previous experience were excluded. The poor association between the number of films reported and passing could be explained by these confounders. It should be noted that the predominant films reported by the residents were chest and limb radiographs, which the majority would have passed. With an almost equal weight given to the truncal region, lack of exposure to films in these domains (save for chest radiographs that were performed not only for diagnosis but also for screening and to complete pre-operative workup) is likely to explain their failing sub-scores on average. This situation may stem from a dearth of performance of such films nowadays or perhaps a lack of confidence, causing residents to shy away from reporting them.<sup>10</sup> Residents should be cognisant that it is not merely the number of films in as much as their variety.<sup>6</sup> Training gaps should be supplemented with additional tutorials and residents taking their own initiative to gain proficiency. Therefore, we postulate that the need to tweak the minimum number is not as crucial (seeing that it had been more than twice surpassed) as compared to the spread of films, which should be implemented at the PD level.

A comparison between the pairs of papers in each attempt provides us with the following insights:

- The likelihood ratio of them passing the 1st attempt if they passed Paper A was 1.87, or an increase of approximately 15%.<sup>15</sup> The findings suggest that a good start in Paper A bodes well, whereas a poor performance may be detrimental enough not to be compensated by better performance in Paper B. A survey by Yeung et al. found that about one-third of respondents felt that anxiety negatively affected their performance in the rapid reporting component of the FRCR 2B exam, despite being the least stressful of the three sections.<sup>11</sup> However, as a stand-alone entity in the PFCA and being their first high-

stakes assessment in residency, the level of perceived anxiety could be much higher. A strategy to alleviate this would involve better preparation in a similar mock setting.<sup>11</sup>

- Error analysis showed a higher percentage of Incorrect Abnormalities for those who passed rather than failed the 1st attempt (approaching significance,  $p=0.064$ ) as well for all repeaters in the 2nd attempt (which was significant,  $p=0.048$ ). The shift towards being classified as an Incorrect Abnormality rather than a False Positive or Negative should be viewed favorably as reflecting a lower tendency to over- or under-call a radiographic abnormality, which can be harder to remediate.

Khan et al., who held their pre-call assessment initially after six months, and in the latter stage after 12 months (rather similar to us), found no difference in performance between cohorts at these time points, but instead discovered an improvement that occurred, at earliest, midway through their second year.<sup>4</sup> Their findings suggest the existence of other factors, such as the spectrum of films and diversity in training, which may accelerate the attainment of higher-order proficiencies, including honing perceptual and cognitive skills required for image interpretation.<sup>16</sup> Similarly, we did not find a significant difference between our residents undergoing short or long remediation, which occurred during RY1. The only way to validate the findings of Khan et al. is to conduct another assessment in RY2. Nevertheless, it is assuring that Tan et al., who audited resident plain film reporting data from our RPs (from 2012 to 2018) found a favorable major discrepancy rate of 0.04-1.13% (mean 0.34%) as compared to American data of 1.4-1.5%.<sup>5</sup>

## LIMITATIONS

Despite the nine-year period of study, the numbers remain small in this niche specialty. Under-estimated numbers may arise from delay entering logbooks, patchy entry once the minimum criteria had been reached, PACS upgrade, or when the dictating radiologist failed to designate the trainee as a co-reader.<sup>17</sup> Ours and future studies would benefit from considering the number of prior films co-reported while rotating through as a MOPEX-MO in radiology.

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# Limitation of cane sugar solution as a tissue fixative for glycogen preservation in mouse liver

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## ABSTRACT

**Introduction:** Sugar-based natural fixatives have been proposed as substitutes for formalin in histopathological practice because of their promising results in preserving tissue structures. However, they have not yet been applied. This study aimed to determine the optimal concentration of cane sugar solution (CSS) for tissue fixation, histochemical staining, and glycogen storage in mouse liver tissues.

**Materials and Methods:** A total of 24 mouse liver tissues from six mice were divided into four groups and fixed with 10% neutral buffered formalin (NBF), 30%, 50% and 70% CSS, respectively, for 24 h at room temperature. After tissue processing and sectioning, the samples were stained with hematoxylin and eosin (H&E), methyl green pyronin Y (MGPY) and peroxidase acid-Schiff (PAS). The tissue sections were evaluated under a light microscope by two blinded pathologists. Data were analyzed using one-way analysis of variance (ANOVA) and a post-hoc test to assess the differences among the experimental groups.

**Results:** The gross morphology of all the samples showed minimal shrinkage without color changes. Microscopic examination revealed that the 50% and 70% CSS groups showed comparable efficacy in cytoplasmic and nuclear staining, H&E staining intensity, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) staining, and collagen staining with the 10% NBF group. The 30 percent CSS group showed significantly poorer nuclear and DNA staining than the 10% NBF group. All CSS groups failed to preserve glycogen in mouse liver tissues ( $p < 0.001$ ) compared with the 10% NBF.

**Conclusions:** Our study concluded that 50% and 70% CSS are potentially replaceable, eco-friendly fixatives for 10% NBF in small tissue samples for tissue fixation and histochemical staining. However, 10% NBF was still superior to CSS for glycogen preservation in mouse liver tissue.

## KEYWORDS:

*Eco-friendly fixatives, glycogen preservation, cane sugar solution, histochemical staining*

## INTRODUCTION

Microscopic evaluation of tissue biopsies has been considered the gold standard for diagnosis for years.<sup>1</sup> Before the tissues can be viewed on a slide, they undergo several processes, of which fixation is the first and arguably the most important one, as it sets the foundation for the subsequent steps. Fixation is a process in which a fixative penetrates the respective tissue, causing physiochemical reactions leading to three main outcomes: prevention of enzyme autolysis, inhibition of bacterial putrefaction, and preservation of cellular components as close to their living state as possible. Since its discovery, formalin has become the most established fixative in the field owing to its several advantages, such as being easily accessible, inexpensive, and having a high degree of accuracy.<sup>2</sup> The most commonly available fixative is 10% neutral buffered formalin (NBF), which crosslinks amino acids and forms methylene bridges.<sup>3,4</sup> Despite its benefits, the major drawback of formalin is its toxicity to human health. Upon contact, it can trigger allergic dermatitis in the skin or irritate the mucous membrane, leading to conjunctivitis.<sup>5</sup> In addition, it has been classified as a group 1 carcinogen due to its ability to cause sinonasal and nasopharyngeal cancers.<sup>6</sup>

Thus, attention needs to be shifted towards effective alternative fixatives that do not threaten human health. Recent studies have shown that natural fixatives such as honey, jaggery, cane, and sugar are comparable to formalin in preserving the tissue and allowing for satisfactory staining and visualization of cellular outline and nuclear morphology.<sup>7-10</sup> These fixatives are non-toxic, eco-friendly, and economical, and do not require special equipment.

Sugarcane is a well-known source of sugar that is rich in sucrose. Various sugarcane products contain 13%–15% sucrose. According to the theory based on the study by Patil et al., sucrose breaking down at low pH produces aldehydes and crosslinks with tissue amino acids, which is similar to the action of formaldehyde in tissue fixation.<sup>8</sup> Moreover, sugarcane has antiseptic/antibacterial properties and cytoprotective activities.<sup>11,12</sup>

In recent years, honey- or sugar-based natural fixatives have become promising alternatives to formalin for tissue fixation because of their sucrose content. Recent studies have shown

This article was accepted: 12 May 2025

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that natural fixatives can fix small tissues without interfering with diagnosis.<sup>13,14</sup> Chittumsetti et al. showed that sugar-based fixatives reveal better nuclear and cytoplasmic staining than formalin.<sup>7</sup> Other studies revealed that sugar-based fixatives were comparable with formalin for special collagen stains, such as periodic acid-Schiff (PAS) and Masson's trichrome, etc.<sup>15</sup> Moreover, sugar-based fixatives revealed promising results in immunohistochemical staining.<sup>16,17</sup> However, formaldehyde is still used in daily clinical practice due to conflicting results among the studies. In addition, no study has been conducted on the detection of nucleic acids using histochemical staining and intracellular glycogen storage.

Therefore, this study aimed to focus on the fixative efficacy of cane sugar solution (CSS) in tissue fixation, morphological preservation, and histochemical staining. Mouse liver tissue morphology was determined using hematoxylin and eosin (H&E), nucleic acid methyl green-pyronin Y (MPGY), collagen staining, and glycogen preservation by peroxidase acid-Schiff (PAS) staining.

## MATERIALS AND METHODS

### Preparation of Cane Sugar Fixatives

Commercially available unrefined raw cane sugar (Country Farms Sdn Bhd, Malaysia) was dissolved in distilled water to prepare 30, 50, and 70% CSS. Commercially available 10% NBF (Thermo Scientific, USA) was used as the control.

### Sample Collection and Fixation

A total of 24 fresh liver tissues from six mouse carcasses were obtained from the IMU University (IMU) research laboratory with the approval of the International Medical University-Joint Committee on Research & Ethics (IMU-JC).<sup>18,19</sup> Fresh liver tissues from each carcass were dissected into 1-cm-thick pieces<sup>20</sup> and immersed into four different fixatives: 30%, 50%, and 70% CSS and 10% NBF with a minimum of 1:10 tissue to fixative volume. The samples were then fixed for 24 hours at room temperature on a shaker. Tissue samples were processed using an automated Tissue-Tek processor with 90-minute per station protocol. Tissues were dehydrated in an ethanol series, cleared with xylene, and infiltrated with molten paraffin. Paraffin-embedded tissues were then sectioned for microscopic examination.<sup>21</sup>

### Histological and Histochemical Staining Evaluation

Commercially available H&E and PAS were purchased from Sigma-Aldrich (M) Sdn. Bhd. For the MGPY solution, 0.5% methyl green solution was prepared by adding 0.025 g of methyl green to 5 ml of 0.2 M acetate buffer at pH 4.2, followed by the preparation of an MGPY solution through the addition of 0.0025 g of pyronin Y to 5 ml of the previously prepared 0.5% methyl green solution.<sup>22</sup> Five- $\mu$ m-thick mouse liver tissues were stained with H&E, MGPY, and PAS according to the manufacturer's protocol. The slides were examined under a light microscope by two pathologists who were blinded to avoid subjective bias. With H&E staining, the tissue sections were assessed for cellular outline, cytoplasmic and nuclear staining, and staining intensity. DNA and RNA staining were assessed using the MGPY stain, whereas glycogen preservation, collagen fiber staining, and nuclear

staining were analyzed using PAS staining. Each criterion was rated on a scale of 1–4 (1 - poor, 2 - satisfactory, 3 - good and 4 - excellent). At least five different fields of view were observed at x400 magnification. Photomicrographs were taken using a Nikon Eclipse 80i light microscope (Nikon Elements version 4.13 software).

### Statistical Analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, version 29; Chicago, IL, USA). One-way analysis of variance (ANOVA) and post-hoc tests were used to assess the differences between the experimental groups. Statistical significance was set at  $P < 0.05$ .

## RESULTS

### Gross Morphology Findings

After 24-hour fixation, all specimens showed minimal shrinkage with no obvious color changes (Figure 1). However, the 30% CSS-fixed tissues produced gas and a fermentation smell after 24 hours of fixation, suggesting sugar fermentation by bacteria.

### Evaluation of Microscopic Morphology and Preservation of RNA and DNA in CSS-Fixed Mouse Liver Tissues

Under microscopic examination, mouse liver tissues fixed in 10% NBF (control) revealed well-preserved nuclei and cytoplasm with distinct cellular outlines (Figure 2a). Thirty percent CSS group showed identifiable cellular and nuclear morphology, with areas of cellular degeneration, cytoplasmic vacuolation, and cellular hyalinization (Figure 2b). Moreover, the 50% and 70% CSS groups showed well-preserved nuclear and cytoplasmic morphology, with a few areas of tissue degeneration. In addition, 70% CSS-fixed tissues showed darkened nuclei, shrunken cytoplasm, and widened sinusoids, suggesting tissue shrinkage compared with the control group (Figure 2c and d).

MGPY was used to evaluate DNA and RNA preservation in mouse liver tissues. It is a cationic dye that stains highly polymerized nucleic acid (DNA) with methyl green and slightly polymerized nucleic acids (RNA) with pyronin Y.<sup>23</sup> The representative picture of the 30% and 50% CSS groups showed low staining intensity and uneven staining patterns compared to the control. The representative image of the 70% CSS group showed similar staining density to that of the control group (Figure 3e–h).

### Evaluation of Glycogen Preservation in CSS-Fixed Mouse Liver Tissues

Next, the preservation of glycogen and collagen was evaluated using PAS staining in the control and CSS groups. None of the CSS concentrations tested was able to stain glycogen, suggesting that CSS failed to preserve glycogen integrity in mouse liver tissues. However, the control and all concentrations of CSS groups exhibited compatible collagen staining. (Figure 3i–l).

**Table I: Analysis of cellular morphology, DNA and RNA staining, collagen staining and glycogen preservation in the control and CSS groups**

Staining methods	Parameters	Control (10% NBF)	30% CSS	50% CSS	70% CSS	p-value	
						One-way ANOVA	Post Hoc Test
H&E	Cellular outline	2.58	1.67	1.83	2.5	0.043*	(Control,70%) vs (30%,50%) p<0.05
	Mean ± SD	(±0.86)	(± 0.26)	(±0.61)	(±0.63)		
	Cytoplasmic staining	2.67	2.00	2.16	2.5	0.396	
	Mean ± SD	(± 0.88)	(± 0.45)	(± 0.98)	(± 0.18)		
	Nuclear staining	3.33	2.00	2.33	2.75	0.019*	Control vs 30% CSS p<0.05
Mean ± SD	(± 0.52)	(± 0.63)	(± 0.98)	(± 0.52)			
MGPY	Staining intensity	3.08	2.50	2.33	2.75	0.306	
	Mean ± SD	(± 0.74)	(± 0.45)	(± 1.08)	(± 0.27)		
	DNA staining	2.67	1.33	1.58	2.42	0.017*	Control vs 30% CSS p<0.05
	Mean ± SD	(± 0.88)	(± 0.26)	(± 0.58)	(± 1.07)		
	RNA staining	3.08	2.00	2.17	2.83	0.71	
Mean ± SD	(± 0.92)	(± 0.45)	(± 0.68)	(± 0.93)			
PAS	Collagen staining	3.25	2.42	2.75	2.67	0.206	
	Mean ± SD	(± 0.76)	(± 0.80)	(± 0.69)	(± 0.26)		
	Glycogen preservation	3.25	0.25	0.25	0.17	<0.001*	Control vs (30% CSS, 50% CSS, 70% CSS) p<0.05
Mean ± SD	(± 1.36)	(± 0.61)	(± 0.42)	(± 0.41)			

Footnote: NBF = Neutral Buffer Formalin, CSS = Cane Sugar Solution, ANOVA = Analysis of variance, H&E = Haematoxylin and Eosin, MGPY = Methyl Green Pyronin Y, PAS = Periodic Acid-Schiff, \*p < 0.05

#### Analysis of Microscopic Morphology, Preservation of DNA, RNA, Collagen and Glycogen in CSS-Fixed Mouse Liver Tissues

The slides underwent a blindfolded assessment independently by two pathologists. The morphological scoring of H&E-stained CSS-fixed tissues was investigated for cellular architecture preservation, and 10% NBF-fixed mouse liver tissues were used as controls. The control group exhibited a mean value of 2.58 for the cellular outline with a standard deviation of 0.86. This value significantly decreased to 1.66 (±0.26) at 30% CSS, slightly increased to 1.83 (±0.61) at 50% CSS and approached the control value again at 2.5 (±0.63) with 70% CSS. Cytoplasmic staining revealed a similar trend. The control group had a mean of 2.67 (±0.88), which dropped to 2.0 (±0.45) at 30% CSS, slightly increased to 2.17 (±0.98) at 50% CSS and was close to the control at 2.5 (±0.45) at 70% CSS. The nuclear staining notably reduced from 3.33 (±0.52) in the control group to 2.0 (±0.63) at 30% CSS. This value modestly increased to 2.33 (±0.98) at 50% CSS and 2.75 (±0.52) at 70% CSS, even though it did not return to control levels. For staining intensity, the control mean was 3.08 (±0.74), which decreased to 2.5 (±0.45) at 30% CSS, further dropped to 2.33 (±1.08) at 50% CSS and then slightly increased to 2.75 (±0.27) at 70% CSS (Table I).

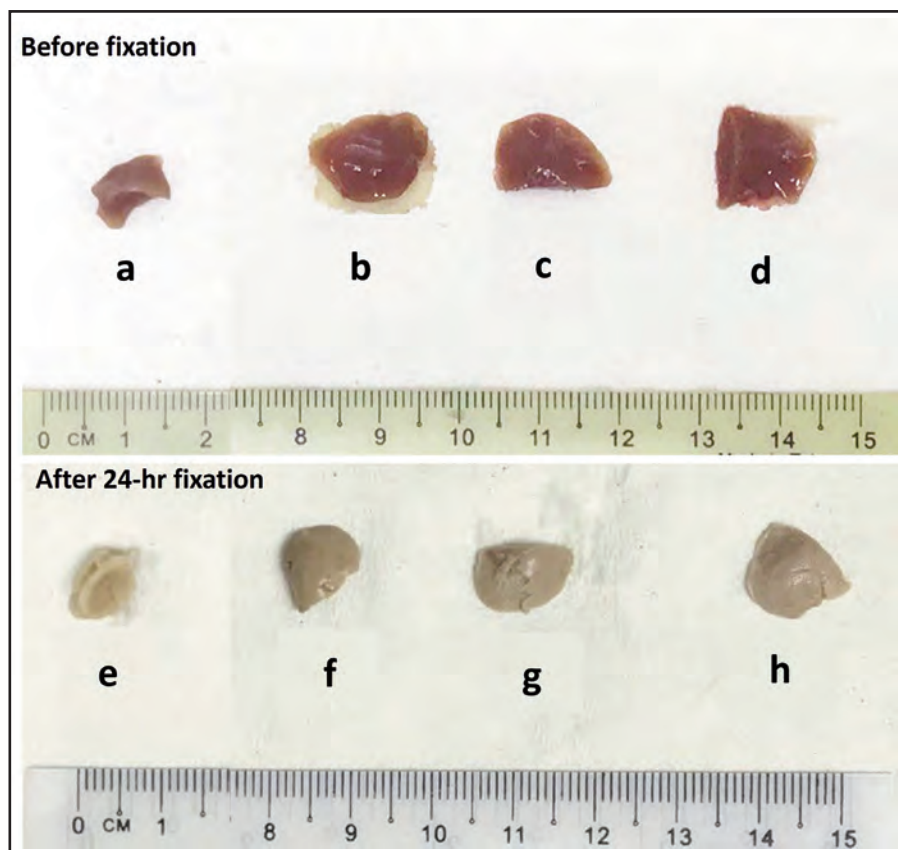
Analysis of the DNA content by MGPY staining revealed that the control group had a mean of 2.67 (±0.88). This significantly decreased to 1.33 (±0.26) at 30% CSS, then slightly increased to 1.58 (±0.58) at 50% CSS and further approached the control value at 2.42 (±1.07) at 70% CSS. The RNA content in the control group had a mean of 3.08 (±0.92). This decreased to 2.0 (±0.45) at 30% CSS, slightly increased to 2.17 (±0.68) at 50% CSS, and further increased to 2.83 (±0.93) at 70% CSS, even though it remained lower than the control (Table I).

The glycogen content stained by PAS showed a dramatic reduction from the control mean of 3.25 (± 1.36) to 0.25 (± 0.61) at 30% CSS. This low level was maintained at 50% CSS with a mean of 0.25 (±0.42) and decreased further to 0.17 (± 0.41) at 70% CSS. The mean collagen level in the control group had a mean of 3.25 (±0.76). This decreased to 2.41 (±0.80) at 30% CSS, then slightly increased to 2.75 (±0.69) at 50% CSS and was 2.67 (±0.26) at 70% CSS, which was still lower than the control value (Table I).

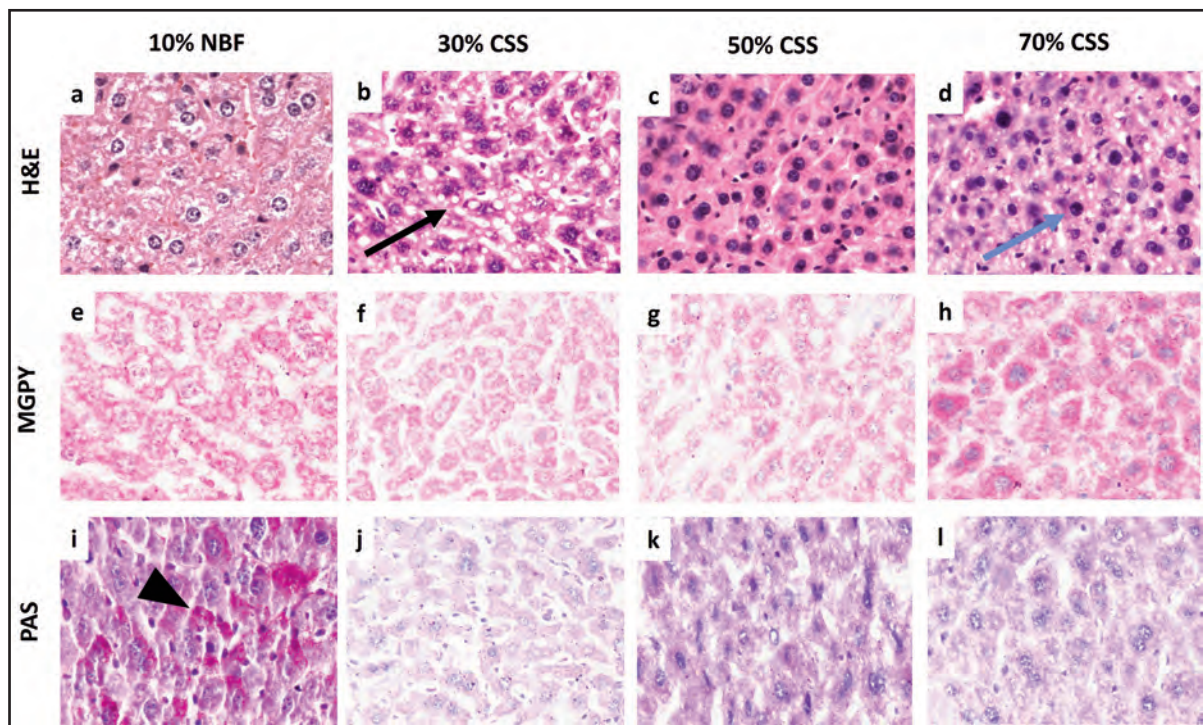
Furthermore, we analyzed the preservation efficacy of CSS using a one-way ANOVA. In H&E staining, the mean cytoplasmic staining and staining intensity of the 30% CSS and 50% CSS groups were lower than those of the control group; however, there was no significant difference among the groups. The cellular outline and staining intensity were significantly different between the groups. Further analysis with the post hoc test revealed that the mean cellular outline was higher in the control and 70% CSS groups than in the 30% and 50% CSS groups. In addition, the 30% CSS group showed significantly poorer nuclear staining than the control group (Table I). Similarly, DNA staining with MGPY in the 30% CSS group showed a significant difference. RNA staining by MGPY and collagen staining by PAS showed no significant differences between the groups. However, glycogen preservation at all concentrations of CSS groups was significantly different from that of the control group (Table I).

#### DISCUSSION

In this study, we investigated the fixative properties of cane sugar solutions in mouse liver tissues using histochemical staining, H&E, MGPY, and PAS staining, and determined the optimal concentration of CSS. Among the three different concentrations (30%, 50%, and 70% CSS), 50% and 70% CSS showed promising fixative efficiency, which can be compared



**Fig. 1:** Gross morphology of mouse liver tissues before fixation (a, b, c, d) and after fixation with 10% NBF (e), 30% CSS (f), 50% CSS (g) and 70% CSS (h)



**Fig. 2:** Mouse liver tissues were fixed with 10% NBF (a, e, i), 30% CSS (b, f, j), 50% CSS (c, g, k) and 70% CSS (d, h, l). H&E stain (a-d): cytoplasmic vacuolation was seen in the 30% CSS group (b, black arrow), 50% and 70% CSS groups (c, d) showed well-preserved cellular morphology, and 70% CSS group (d) showed darkened nuclei (blue arrow) compared to 10% NBF group (a). MGPY stain (e-h): 30% and 50% CSS groups (f, g) showed low staining- intensity in DNA and RNA staining. PAS stain (i-l): 10% NBF group showed well-preserved glycogen in the cytoplasm of mouse liver (i, black arrowhead), while 30%, 50%, and 70% CSS groups failed to preserve glycogen (j-l)

with formalin in terms of cellular morphology and DNA, RNA, and collagen preservation. However, all CSS concentrations failed to preserve glycogen in the mouse liver tissues.

Despite being the most widely used ideal fixative for more than a century, 10% NBF has been associated with several significant drawbacks: hardening and discoloration of tissues, unpleasant and irritating odor, and formation of formic acid, which is corrosive and poses serious health risks to laboratory personnel upon exposure.<sup>24,25</sup> Moreover, 10% NBF remains popular in routine laboratory practice due to its low cost, ability to quickly fix large amounts of tissue, and germicidal properties that prevent autolysis and putrefaction.<sup>24</sup> However, health and environmental concerns have driven the search for safer alternatives.

Natural products such as sugar and honey have been used in the food industry as natural preservatives to extend the shelf life of various foods owing to their properties, such as inhibition of microbial growth, reduction of water activity, and preservation of color, texture, and flavor. Higher fructose content in sugar, jaggery, and honey suggests a potential fixation mechanism by converting fructose to aldehyde and creating a cross-link with tissue amino acids.<sup>8</sup>

Our study revealed that 50% and 70% CSS had potential fixative effects on mouse liver tissues and their histochemical staining, whereas 30% CSS failed to preserve these tissues. Our findings are consistent with those of Udonkang et al. (2018), who observed that tissue fixed in a higher concentration of a honey solution (70% and above) showed similar nuclear and cytoplasmic staining qualities to formalin-fixed tissues in 48-h to 6-month of fixation.<sup>16</sup> Jaggery, a sugarcane derivative, is compromised with a high content of fructose, glucose, and 20% water. The high osmolarity of sucrose solution causes water to be drawn out of the tissue, leading to dehydration. This dehydration helps to fix and preserve the tissue morphology by preventing swelling and distortion.<sup>26</sup> Aligned with these findings, we observed that CSS-fixed tissues were more dehydrated than 10% NBF-fixed tissues, which led to the challenge in microtome sectioning. However, it did not affect the morphology of mouse liver tissues or the further staining process. The concentration of CSS differs from previous studies in which lower concentrations (20–30%) showed a promising fixative effect on tissue preservation.<sup>7,8,10,26,27</sup> Previous studies on the fixative activity of sugar indicated that a concentration of approximately 195 g of sugar per 100 g of water is needed to achieve a water activity level that is sufficient to inhibit the growth of bacteria.<sup>28,29</sup> Although fixation duration was 24 h in all studies, sucrose and glucose composition and additional components might be different among these studies and might affect fixative efficiency.

Majumdar et al. (2016) reported that the reaction of reducing sugars and amino acids in an acid medium or under thermal stress led to a Maillard reaction, which resulted in an intermediate product, a furanic compound. The furanic compound was further accelerated into a hydroxymethylfurfural (HMF) compound with an aldehyde group. The fixation process involves the cross-linking of the

proteins present in the tissues by HMF through a di-Schiff base reaction. Nucleic acid fixation may also occur by the reaction of MHF with the free amino groups of nucleotides.<sup>30</sup> MGPY is a classic histological stain that uses basic dyes to differentiate between DNA and RNA. Methyl green binds to phosphate radical in the DNA double helix to stain it green-blue, whereas pyronin Y binds to RNA to stain it red.<sup>31</sup> As first evidence, we showed that 30% CSS failed to preserve, but 50% and 70% CSS preserved the nucleic acid, similar to formalin by MGPY staining. High sugar concentrations in CSS create a hypertonic environment that reduces water activity and inhibits the activity of nucleases that can degrade nucleic acids.<sup>8,32</sup>

Interestingly, our findings revealed that all concentrations of CSS failed to preserve glycogen in the mouse liver tissues. It has been reported that glycogen is not directly fixed, but is trapped with fixed proteins.<sup>33,34</sup> The penetration rate of formaldehyde in 10% NBF is approximately 1 mm per hour for the first hour, followed by a slower rate of about 1 mm every 3 hours. While specific quantitative data on the penetration rate of CSS fixatives are less documented, it is possible that CSS may have a slower penetration rate into the tissue, which leads to protein digestion and results in an inability to preserve glycogen.

This study demonstrated that CSS at concentrations of 50% and 70% offers comparable efficacy to 10% NBF in preserving tissue morphology, nucleic acids, and collagen in small tissue samples. These findings suggest that CSS is a viable and safe alternative to formalin for short-term fixation, particularly for small tissue specimens. However, it is important to acknowledge the limitation that CSS is ineffective in preserving glycogen. Further research is needed to determine whether this limitation can be addressed through modified fixation protocols or if alternative sugar solutions might offer better glycogen preservation. In addition, the long-term stability of tissues fixed with CSS compared to those fixed with 10% NBF remains unclear. Moreover, it is important to address the technical challenges encountered in CSS-fixed tissue. Common artifacts such as scratch lines appearing in sections, opaque sections, and tissue-block separation during microtomy are frequently observed. The dehydrating properties of CSS render the tissue brittle, likely causing over-dehydration and resulting in artifacts during sectioning.<sup>35</sup>

Overall, CSS holds promise as a safe and effective fixative for specific applications, particularly for short-term fixation of small tissue samples where glycogen preservation is not a critical factor. However, further studies are required to fully define its suitability for broader use in tissue pathology.

While providing insights into the use of CSS as a tissue fixative, this study had several limitations. This study focused solely on mouse liver tissue, and the results may not be applicable to other tissue types of species. A recent study revealed that tissue fixation at 4°C with 80% alcohol showed better glycogen preservation compared to fixation at RT.<sup>36</sup> The fixation time was fixed at 24 h at RT, and exploring different fixation durations and temperatures could reveal optimal conditions for tissue fixation and glycogen preservation. This study did not directly address the clinical

relevance of CSS as a fixative. Further research is required to determine whether CSS can be effectively used for diagnostic pathology.

#### ACKNOWLEDGMENT

This study was funded by the IMU University Biomedical Science Program {BMSc I-2020(03), BMSc I-2022(11)}, and Bachelor of Medical Science Program {BMS I-2022 (10)} to Phyu Synn Oo.

We thank Associate Professor Dr. Kenny Voon Gah Leong (University of Nottingham, Malaysia), Dr. Jestin Chellian (IMU University), Wee Hong Zhuang, and Cindy Yeoh Shin Ly (Biomedical Science Programme, IMU University) for their help with animal experiments, and Associate Professor Dr. Akil Daher (IMU University) for his consultancy in data analysis.

#### CONFLICT OF INTEREST

There is no actual or potential conflict of interest in relation to this article.

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# Human T-lymphotropic virus-1 proviral load among patients on maintenance hemodialysis

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## ABSTRACT

**Introduction:** Human T-lymphotropic virus type 1 (HTLV-1) is a complicated leukemogenic retrovirus, it is the first oncogenic virus discovered and identified as the leading cause of T-cell malignancy and HTLV related myelopathy with a long latency period. Transmission via infected blood products is one of the common routes of HTLV-1 infection, and hemodialysis patients are potentially more vulnerable to blood-borne viral infections such as HTLV. This study aimed to determine the factors associated with HTLV-1 proviral load among hemodialysis patients.

**Materials and Methods:** A cross-sectional study of 130 patients undergoing hemodialysis was conducted between November 2023 and January 2024. After HTLV-1 RNA extraction, complementary DNA (cDNA) was synthesized. HTLV-1 pro-viral load (PVL) was quantified by real-time PCR (qRT-PCR).

**Results:** HTLV-proviral load was detected in 57 (43.84%) blood samples, with a mean  $\pm$  standard deviation (SD) of  $16.3 \times 10^3 \pm 5.4 \times 10^4$  (copies/ $\mu$ L), including 34 (26.2%) males and 23 (17.7%) females. The highest percentage of infection (22.3%) was detected in the 40-59 age group and married patients (39.2%); however, a high viral load was observed in the 20-39 age group. Frequent blood transfusions associated with HTLV-1 infection were observed in 42 patients (32.3%). Myelopathy-related muscle spasms and paresthesia were significantly associated with the HTLV-1 viral load ( $p < 0.05$ ). A statistically significant relationship was observed between asymptomatic patients and high HTLV-1 proviral load ( $p = 0.004$ ).

**Conclusion:** These findings show that a significant number of dialysis patients were infected with HTLV-1. Therefore, hemodialysis patients should be frequently screened for HTLV-1 infections.

## KEYWORDS:

HTLV-1, hemodialysis, real-time PCR, oncovirus, myelopathy

## INTRODUCTION

Human T-lymphotropic virus Type One (HTLV-1) belongs to the Retroviridae family and is classified into the genus Deltaretrovirus.<sup>1</sup> HTLV-1 has a genome of positive-sense dimeric single-stranded RNA similar to other retroviruses,

which encodes structural and enzymatic proteins such as gag, env, and pol, as well as a unique region at the 3' end, referred to as the pX region.<sup>2</sup> This indicates the presence of regulatory proteins such as Rex, basic leucine zipper factor (HBZ), and trans-activator protein (Tax).

Globally, approximately 10 to 20 million people are reported to be carriers of HTLV-1, with the virus being more prominent in specific countries such as Japan (2.7%-21.2%), the Caribbean region (up to 6%), Brazil (up to 2%), Northeast Iran (0.6%), Central Africa, and many regions of South America.<sup>3,4</sup>

HTLV-1 transmission in vivo is facilitated by breastfeeding, blood transfusions, needle sharing, and sexual contact.<sup>5,6</sup> Furthermore, HTLV-1 has been shown to spread efficiently in transplant patients.<sup>7</sup> During hemodialysis (HD), the blood is filtered through a dialysis machine. Although this procedure can be effective in treating kidney failure, it also exposes patients to the danger of infectious complications of hemodialysis, including infections caused by contaminated water or equipment, and the transfer of various blood-borne viruses, such as HIV, HTLV, and HCMV.<sup>8,9</sup> Patients undergoing HD are among the highest risk categories for HTLV-1 transmission because they regularly require blood transfusions,<sup>10</sup> and HD patients, as well as those with thalassemia and hemophilia, are more vulnerable to infections acquired through transfusions.<sup>11</sup> The relationship between HTLV-1 transmission and blood transfusion is widely recognized and has been validated in various investigations of blood donors from several countries.<sup>12-14</sup>

Previous research has shown that individuals infected with HTLV-1 have a higher adjusted risk of death from any cause compared to those who are HTLV-1 negative, with a relative risk of 1.57 (95% CI 1.37–1.80).<sup>15</sup> Additionally, these individuals are at increased risk of developing diabetes and chronic kidney disease.<sup>16</sup>

HTLV-1 is a known cause of adult T-cell leukemia (ATL), which develops in 5% of affected individuals, as well as a life-threatening neurological condition, HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP). Moreover, various inflammatory disorders associated with this infection have been documented, including uveitis, Hashimoto thyroiditis, Graves' disease, pulmonary disease, infective dermatitis, myositis, and arthritis.<sup>17</sup>

This article was accepted: 28 May 2025

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HTLV-1 infection causes continuously elevated levels of proinflammatory cytokines and chemokines systemically<sup>18,19</sup> which have been independently linked to accelerated renal function loss in other cases.<sup>20</sup> A study conducted in Brazil found that 2 out of 15 individuals who tested positive for HTLV (approximately 13.3%) experienced bladder dysfunction. This may be attributed to neurological impairment caused by the virus, which could contribute to the development of end-stage renal disease. However, a definitive clinical link between HTLV infection and this disease has not yet been established.<sup>21</sup>

A recent assessment surveyed the status of hemodialysis patients in Iraq, involving 10,721 cases from all Iraqi governorates. The study found that Baghdad had the highest population of patients undergoing hemodialysis, accounting for 27.0% of the cases. Additionally, 71.8% of the patients received two hemodialysis sessions each week. Hypertension and diabetes mellitus were identified as the primary etiological factors leading to renal disease in these patients, affecting 40.3% and 32.3% of these patients, respectively.<sup>22</sup> Due of the increasing spread of this virus worldwide and limited epidemiological data, this study aimed to investigate HTLV-1 infection in hemodialysis patients and to evaluate the associated factors.

## MATERIALS AND METHODS

A cross-sectional study was conducted involving 130 hemodialysis patients of both sexes, aged 18–82 years, who had been undergoing hemodialysis for at least six months due to longstanding, uncontrolled diabetic nephropathy with or without hypertension. Participants were recruited from the Dialysis Center at Al-Imamein Kadhimein Medical City in Baghdad, Iraq, between November 2023 and January 2024. Patients under 18 years of age were excluded from the study, as many declined to participate or had not attended the center regularly. Additionally, HIV-infected patients were not included because HIV can activate HTLV-I viral expression through interactions with host cellular genes, potentially affecting the accuracy of proviral load results.

The Ethics Committee of the Department of Microbiology at the College of Medicine, University of Baghdad approved the research protocol (IRB No. 0230A in November 2023). After being fully informed about the study objectives, all patients signed a consent form for participation.

Each patient was interviewed and data regarding their age, sex, history of chronic diseases, blood transfusion, and details about their dialysis conditions such as vascular access, cause, frequency, and duration of dialysis were collected.

A five-milliliter whole blood sample was taken from each patient aseptically via venipuncture. Four hundred microliters of blood was then mixed with 600  $\mu$ L of triazole reagent in Eppendorf tubes and stored at  $-20^{\circ}\text{C}$  until further use. Sample collection was performed with the consent of all the participating patients.

## RNA extraction

HTLV-1 RNA was extracted from each blood sample using the organic method outlined in the TRIzol reagent protocol. Specifically, 0.4 mL of blood was combined with 0.6 mL of TRIzol™ Reagent, and the lysate was homogenized by carefully pipetting up and down several times. To each tube, 0.2 mL of chloroform was then added, and the tube cap was securely fastened. These mixtures were incubated for 2–3 minutes before centrifugation for 10 minutes at 10000 rpm. This process resulted in the separation of the mixture into a lower organic phase, interphase, and colorless upper aqueous phase. The aqueous phase containing the RNA was subsequently transferred to a new tube and stored at  $-20^{\circ}\text{C}$  for later analysis. To precipitate total RNA, isopropanol (0.5 mL) was added to the aqueous phase, and the mixture was incubated for an additional 10 min before being centrifuged for 10 min at 12,000 rpm. Finally, the supernatant was discarded, leaving a white gel-like pellet at the tube bottom. The RNA was washed by adding 70% ethanol (0.5 mL), vortexed briefly, and centrifuged for 5 minutes at 10000 rpm.

## cDNA synthesis

Complementary DNA (cDNA) was synthesized from an RNA template using OT-1 Reagent Kits (Reverse Transcription Kits), produced by Synthol, Russia. Throughout the procedure, the manufacturer's protocol strictly adhered to each stage of the reaction. cDNA synthesis involved two thermal cycling reactions. In the initial reaction, a random primer and the isolated RNA were subjected to a thermal cycle consisting of 5 min at  $70^{\circ}\text{C}$ , followed by 10 min at  $4^{\circ}\text{C}$ . The subsequent cDNA reaction was conducted under the following conditions: 15 min at  $37^{\circ}\text{C}$ , 1 h at  $45^{\circ}\text{C}$ , and concluded with 5 min at  $92^{\circ}\text{C}$ . As specified by the manufacturer, the total reaction volume was maintained at 20  $\mu$ L. The concentration of cDNA was quantified using a Quantus Fluorometer, revealing concentration values within the range of 0.054–15 ng/ $\mu$ L.

## Real time PCR (RT-PCR)

Quantitative Real-Time PCR (SYBR green assay) was performed using a Mic qPCR Cycler (Bio Molecular System) employing GoTaq qPCR Master Mix (Promega, USA). To detect HTLV-1, primer sequences supplied by (Macrogen/Korea) targeting the virus transactivating gene (Tax gene) were used as follows: forward primer, 5'CCCTAATAATTCTACCCGAGGACTG3' and reverse primer, 5'GCCATCGGTAAATGTCCAAATAAGG3.'

The procedure was carried out in a reaction volume of 10  $\mu$ L according to the instructions provided by the manufacturer. The thermal cycler reactions were programmed as follows: one cycle of initial denaturation was set at  $95^{\circ}\text{C}$  for 5 min, followed by 40 cycles of denaturation, annealing, extension was set at  $95^{\circ}\text{C}$  for 20 min,  $63^{\circ}\text{C}$  for 20 min, and  $72^{\circ}\text{C}$  for 20 minutes respectively. A standard curve was created using sequential dilutions of the template version number used in the qPCR assay. Linear regression of log concentration (copy  $\mu$ L<sup>-1</sup>) versus CT gives the standard curve, and proviral load was expressed as HTLV-1 (copy/ $\mu$ L) peripheral blood.

Table I: HTLV-1 viral load in relation to the patients' characteristics

Variables	Positive HTLV-1 No. (%)	Negative HTLV-1 No. (%)	95% confidence interval	Viral load (Copies/ $\mu$ L) Mean $\pm$ SD	P-value
Age/year:					
<20	2 (1.5)	1(0.8)	0.16-0.30	$2.6 \times 10^3 \pm 3.6 \times 10^3$	0.63
20-39	8(6.2)	11(8.5)		$33.73 \times 10^4 \pm 8.2 \times 10^3$	
40-59	29(22.3)	30(23.1)		$11.6 \times 10^3 \pm 5.6 \times 10^4$	
$\geq 60$	18(13.8)	31(23.8)		$13.6 \times 10^3 \pm 4.8 \times 10^4$	
Sex:					
Male	34(26.2)	43(33)	0.19 - 0.34	$13.5 \times 10^3 \pm 5.6 \times 10^4$	0.93
Female	23(17.7)	30(23.1)		$20.5 \times 10^3 \pm 5.2 \times 10^4$	
Marital status:					
Married	51(39.2)	63(48.5)	0.31-0.47	$6.4 \times 10^3 \pm 12.2 \times 10^4$	0.34
Unmarried	6 (4.6)	10 (7.7)		$56.6 \times 10^3 \pm 13.3 \times 10^4$	
Diabetes Miletus					
Yes	26(20)	26(20)	0.13 - 0.27	$24.8 \times 10^2 \pm 3.5 \times 10^3$	0.82
No	31(23.8)	47(36.2)			
Hypertension					
Yes	46(35.4)	60(46.1)	0.27 - 0.43	$12.6 \times 10^3 \pm 3.8 \times 10^4$	0.24
No	11(8.5)	13(10)			
Total No. (%)	57(43.8)	73(56.2)	0.35-0.52	$16.3 \times 10^3 \pm 5.4 \times 10^4$	0.8

Table II: HTLV-1 RT-PCR results in relation to hemodialysis risk factors

Risk factor	Positive HTLV-1 NO.(%)	Negative HTLV-1 NO.(%)	95% confidence interval	Viral load (Copies/ $\mu$ L) Mean $\pm$ SD	P-value
Frequency of blood transfusion					
Nil	16(12.3)	19(14.6)	0.18 -0.33	$2.78 \times 10^3 \pm 0.33 \times 10^4$	0.63
Once	8(6.2)	15(11.5)		$36.66 \times 10^3 \pm 7.38 \times 10^4$	
More than two	33(25.4)	39 (30)		$17.92 \times 10^3 \pm 6.15 \times 10^4$	
Vascular access					
AV fistula	51(39.23)	64(49.23)	0.31 -0.47	$16.3 \times 10^3 \pm 5.4 \times 10^4$	0.74
Double lumen	6(4.62)	9(6.92)		$14.9 \times 10^3 \pm 4.1 \times 10^4$	
Frequency of dialysis/week					
Twice	15(11.53)	10(7.7)	0.24 - 0.40	$17.3 \times 10^3 \pm 5.6 \times 10^4$	0.07
Three time	42(32.3)	63(48.5)		$10.74 \times 10^3 \pm 3.5 \times 10^4$	

Table III: HTLV-1 RT-PCR results in association to myelopathy clinical presentations

*Symptoms	HTLV-1 PCR results		95% confidence interval	P-value
	Positive No. (%)	Negative No. (%)		
Progressive weakness				
Yes	6 (4.6)	11(8.5)	0.02 - 0.09	0.45
No	51(39.2)	62(47.7)		
Muscle spasm				
Yes	49 (37.7)	44(33.8)	0.29 - 0.46	0.001
No	8(6.2)	29(22.3)		
Paresthesia				
Yes	40 (30.8)	38(29.2)	0.23 - 0.39	0.03
No	17(13.1)	35(26.9)		
Asymptomatic				
Yes	5 (3.8)	21(16.2)	0.01-0.08	0.004
No	52(40)	52(40)		

\*Patients may experience more than one symptom

### Statistical analysis

Data were analyzed using IBM's Statistical Package for the Social Sciences, version 21 (SPSS). Data were compared using Pearson's chi-square ( $\chi^2$ -test) for the two groups and ANOVA for multiple variables. Statistical significance was set at  $p < 0.05$ .

### RESULTS

Out of the enrolled 130 patients predominantly male (77 males and 53 females) aged 18 to 82 years, the mean age  $\pm$  SD was  $51.1 \pm 14.8$  years. The male-to-female ratio was 1.5:1. HTLV-1 proviral load was identified in 57 (43.8%) patients, with a mean proviral load  $\pm$ SD of  $16.3 \times 10^3 \pm 5.4 \times 10^4$ , 34 (26.2%) were male and 23 (17.7%) were female. Males were observed to have higher HTLV-1 infection rates. Data obtained from this study revealed that 26 (20%; 95%CI 0.13-

0.27) of diabetic patients were HTLV-1 infected individual, and 46 (35.4%; 95%CI 0.27-0.43) of patients had hypertension. No statistical difference was observed between HTLV-1 viral load and any other chronic disease, as shown in table I.

In this study, 33 patients (25.4%; 95%CI 0.18 -0.33) with a viral load of who received frequent blood transfusions had a positive viral load of  $17.92 \times 10^3 \pm 6.15 \times 10^4$  compared to 16 (12.3%) with a viral load of  $2.78 \times 10^3 \pm 0.332 \times 10^4$  did not receive any blood. Regarding dialysis vascular access, fifty-one (39.23%;95%CI 0.31-0.47) of patients who were on arteriovenous (AV) fistula showed positive evidence of HTLV-1 infection, while 6 (4.62%) were using double lumen as hemodialysis access; according to the study results, the majority of HTLV-1 positively detected patients were observed in those who underwent hemodialysis three times per week 42(32.3%;95%CI 0.24 - 0.40), as illustrated in Table II.

Human T-lymphotropic virus-related myelopathy was also identified in this study, and the results demonstrated that HTLV-1 was associated with muscle spasm in 49 (37.7%) and paresthesia in 40(30.8%) patients. Significant statistical differences were found in HTLV-1 RT-PCR results ( $p < 0.05$ ). A highly significant association of asymptomatic patients with HTLV-1 results was observed with a p-value of 0.004, as shown in Table III.

## DISCUSSION

HTLV-1 infection has spread globally, the scarcity of recent epidemiological studies, and the majority of HTLV prevalence data are mainly derived from endemic areas; hence, the real number of HTLV-1 infection rates is probably greater than previously reported.<sup>23</sup> Patients undergoing hemodialysis have a greater risk of infection, which can be attributed to weakened immune systems, frequent hospital stays, and surgeries.

This research is a complementary study to our previous work that focused on the detection of HTLV-1 P24 antigen indicating a significant active antigenemia among hemodialysis patients in 60/130(46.2%) of cases,<sup>24</sup> these findings were almost similar to the present study. Notably, in the current study of HD patients, HTLV viral load was detected in 43.84% of them, indicating a high frequency of infection. There is limited information on HTLV-1 prevalence and its associated diseases in Iraq. However, earlier studies have reported the presence of HTLV-1 in patients with lymphoma and leukemia and among blood donors. HTLV-1 and HTLV-2 were first investigated in patients with leukemia and lymphoma in Iraq between July 2008 and May 2009. The research found that 3 out of 250 patients studied had a positive result, resulting in a prevalence rate of 1.2%.<sup>25</sup> Additionally, in 2015, a study was conducted to screen 15,239 Iraqi blood donors for antibodies to HTLV-1 and HTLV-2 in their serum, using a chemiluminescent microparticle immunoassay for confirmation. The results revealed that Thirty-nine donors tested positive for either HTLV-1 or HTLV-2. Prevalence rates varied by region, with the highest occurrence in Baghdad, where 24 individuals (61.5%) were seropositive. Other regions reported the following positive

cases: Karbala, five (12.8%); Al-Qadisiyyah, four (10.2%); Al-Najaf, two (5.1%); Al-Muthanna, two (5.1%); Al-Basrah, one (2.5%); and Wasit province, one (2.5%).<sup>26</sup>

HTLV-1 was more prevalent among individuals aged 40–59 years, suggesting prolonged exposure to the virus. However, high viral loads have been observed at an early age, indicating the possibility of sexual transmission, particularly in those below the age of 40 years. In comparison to the results of a previous study in Iran, HTLV-1 seropositivity was recognized in 20 cases, representing 14.5% of the total, and all positive results were verified by the line immunoassay, 10.5% were males and 19.3% females, and the incidence rate of HTLV-1 infection was significantly higher in older patients aged 65 years or above (25.58%.<sup>27</sup> Moreover, in a cross-sectional study of maintenance hemodialysis (MHD) patients in Brazil, HTLV-1 infection was found to be (2.48% (15/605). The incidence of HTLV-1 infection increased with age, from 1.6% in individuals aged 18 to 30 years to 2.9% in those over 50 years of age. The chance of being positive for HTLV-1 increases by 4% in each year of age. The fact that aging increases exposure to events such as blood transfusions, intravenous drug use, and sexual activity, which can lead to the virus's acquisition, could be one explanation for this association. HTLV-1 requires no known medical treatment. Unfortunately, once the infection is proven, it is passed throughout life.<sup>28</sup> Infection prevention is therefore critical to contain it.

Our findings indicate that males had a higher infection rate than females, with 36.2% of HTLV-1 infected patients being males. Additionally, married patients exhibited a higher percentage of infection than unmarried patients, despite the latter having a higher viral load. This observation contrasts with previous cross-sectional studies among hemodialysis patients, which reported higher rates of HTLV-1 infection in females than in males and unmarried individuals.<sup>29</sup>

In the present study, 33 of 130 hemodialysis (HD) patients (25.4%) who received frequent blood transfusions tested positive for HTLV-1 viral load compared to those who did not receive transfusions. Because of the frequent need for blood transfusions, patients undergoing HD are among the highest risk groups for HTLV-1 infection<sup>10</sup>, Previous research has established this relationship, highlighting the potential risk associated with transfusing blood infected with HTLV-1. One study found that the incidence of infection was significantly higher in dialysis patients who had received blood transfusions, with 153 out of 681 (22.5%) testing positive for HTLV-1, than in those who had never received transfusions.<sup>30</sup>

The process of hemodialysis itself involves regular and extended blood exposure through vascular access and the extracorporeal circle, as well as the proximity of other patients during dialysis, interaction with medical personnel, and replacement of dialysis equipment.<sup>31</sup> According to this study, 38.23% of patients with AV fistula were HTLV-1 positive; furthermore, patients who underwent dialysis three times per week experienced the highest rate of infections. Infection is another significant concern that typically involves vascular access<sup>32</sup> as 20 percent of AV fistula complications are caused by infection.<sup>33</sup>

The development of effective and affordable HTLV screening strategies for donated blood samples should be carefully evaluated in prevalent area. Thus, it is likely to have a substantial impact on the occurrence of HTLV-associated diseases. HTLV screening of donated blood has been routinely implemented in Brazil, Canada, the United States, and other countries in the United States. This certainly reduced the incidence of new infections among blood recipients.<sup>34</sup>

Moreover, 20% of HTLV-1 positive hemodialysis patients in the current study had diabetes. The strong relationship between HTLV-1 and long-term inflammation, as well as the connection between this viral life cycle and glucose metabolism, could be responsible for these disease correlations. According to an Australian study, increased HTLV-1 PVL was linked to a two-fold rise in the incidence of diabetes and chronic kidney disease.<sup>17</sup> HTLV-1 virology is inextricably linked to glucose metabolism, and individuals with high HTLV-1 viral load are more likely to develop diabetic comorbidities, including chronic kidney disease. Diabetes was shown to be more common among Australian Aboriginal people in a study of dialysis patients.<sup>34</sup> *in vitro*, HTLV-1 infection decreased glucose transporter 1 (GLUT-1) expression on the plasma membrane. This was related to inadequate glucose absorption.<sup>35,36</sup>

Most individuals infected with HTLV-I remain asymptomatic carriers throughout their lives, with only 5-10% developing noticeable clinical symptoms. In cases related to human T-lymphotropic virus-associated myelopathy, our findings suggest a close link between HTLV-1 infection and symptoms, such as muscular spasms and paresthesia. Additionally, HTLV-1 triggers a strong inflammatory response in the human body, which may increase the risk of non-communicable diseases. Once infected, the virus causes CCR4+ CD4+ T cells to produce pro-inflammatory cytokines such as interferon- $\gamma$ , leading to the establishment of a T-helper1 phenotype *in vivo*. Elevated levels of pro-inflammatory cytokines and chemokines in both the bloodstream and tissues are believed to contribute to organ inflammation, including the central nervous system.<sup>17,18</sup> This inflammation is closely correlated with a higher HTLV-1 proviral load in peripheral blood cells.<sup>37,38</sup>

This study has some limitations that are important considerations. A primary limitation of cross-sectional research is that correlations do not imply causation, especially because this study was conducted at a single center. Additionally, the small sample size may have affected the accuracy of the estimates for viral percentages. Nevertheless, the quantification of viral copies using qPCR is highly sensitive and can effectively predict associated diseases. It is important to note that patients with very low proviral loads may give false-negative results; however, these individuals are unlikely to transmit the virus because of their extremely low viremic status.

## CONCLUSION

The incidence of HTLV-1 infection in Iraqi hemodialysis patients is significantly high. Therefore, early detection at dialysis centers is critical for minimizing the risk of HTLV-1

infection. Therefore, preventive measures are mandatory for infection control. Furthermore, investigations across various Iraqi cities are recommended for other high-risk populations.

## ACKNOWLEDGEMENT

The authors extend their sincere appreciation to all the HD patients who participated in this study.

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# Determinants of measles cases with complications in Selangor, Malaysia from year 2014 to 2023 using e-Measles registry

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## ABSTRACT

**Introduction:** Measles is a highly contagious vaccine-preventable disease endemic to Malaysia. This study aimed to determine the determinants of measles cases with complications in Selangor, Malaysia, from 2014 to 2023.

**Materials and Methods:** This was a cross-sectional study of confirmed measles cases in Selangor using the secondary surveillance database, Sistem Maklumat Siasatan Measles (SM2). The data were reviewed from April 2024 to June 2024. All registered measles cases in Selangor from 2014 to 2023 were analyzed to determine the determinants of measles cases with complications, the type of measles complications, and the characteristics of those who developed measles complications.

**Results:** Of 3074 measles cases, 479 (15.6%) developed measles complications, of which diarrhea was the most common complication (87.6%), followed by pneumonia (11.6%), otitis media (0.6%), and subscloping panencephalitis (0.2%). The majority of those who developed measles complications were aged less than one year old (42.1%) and 1–6 years (31.4%). The significant determinants of measles cases with complications were non-Malaysian nationality (aOR: 2.1, 95% CI: 1.44, 3.05), cases from urban areas (aOR: 1.8, 95% CI: 1.22, 2.76), cases that fulfilled the measles clinical case definition (aOR: 2.1, 95% CI: 1.59, 2.76), cases with a history of contact with other measles cases (aOR: 4.4, 95% CI: 3.43, 5.67), cases from the measles outbreak (aOR: 1.5, 95% CI: 1.10, 2.19), and cases that were hospitalized (aOR: 5.6, 95% CI: 4.49, 7.07).

**Conclusion:** Strengthening immunization coverage, particularly among high-risk groups, such as non-Malaysians and young children, is essential. Enhanced surveillance, targeted health education, and improved outbreak response strategies are crucial to reduce measles complications in the community. Further research is needed to explore additional factors contributing to measles complications and to assess the effectiveness of intervention measures.

## KEYWORDS:

*Measles, measles cases with complication, registry, hospitalization, Selangor*

## INTRODUCTION

Measles is a highly contagious acute viral disease characterized by fever, maculopapular rashes, cough, runny nose, and conjunctivitis.<sup>1,2</sup> Despite the availability of a safe and effective vaccine, measles remains a significant global health threat, particularly in regions with low vaccination coverage.<sup>3</sup> The disease primarily affects unvaccinated or under-vaccinated children under five years old, leading to severe complications such as diarrhea, pneumonia, otitis media, encephalitis, and blindness.<sup>3</sup> In 2021 and 2022, measles caused an estimated 128,000 and 136,000 deaths, respectively, worldwide.<sup>3</sup> The recent surge of 56,634 cases and four deaths in the WHO European Region in early 2024 highlights the ongoing risk exacerbated by disruptions to routine immunization and healthcare services during the COVID-19 pandemic.<sup>4</sup> Strengthening vaccination programs and ensuring healthcare access are crucial to achieving measles elimination.<sup>5</sup>

Previously, Malaysia had proposed the elimination of measles by the year 2010, but the target year for elimination was pushed to 2025 due to increased measles incidence from 6.1 per million population in 2013 to 52.1 per million population in 2017.<sup>6</sup> There was increased incidence of measles especially in Selangor from 3.4 per million population in 2021 to 73.1 per million population in 2023.<sup>7</sup> A higher incidence of measles will result in a higher risk of complication, particularly in children and immunocompromised individuals.<sup>8</sup> Identifying factors that contribute to measles cases with complications help to comprehend the overall impact of measles on public health and provide insight into the overall burden of the disease in Selangor.

While previous studies in Malaysia have primarily focused on the incidence and outbreak of measles, research on the specific determinants of measles cases with complications, particularly in urban settings and among non-Malaysian populations, is limited. Given the increasing trend of measles incidence in Selangor, this study aimed to determine the determinants, trends, and types of measles cases with complications in Selangor, Malaysia, from 2014 to 2023. Identifying the determinants of measles cases with complications will help in early detection and management of the disease, leading to better patient outcomes.

This article was accepted: 28 May 2025

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## MATERIALS AND METHODS

### Study Design

This cross-sectional study used secondary data obtained from e-Measles, also known as Sistem Maklumat Siasatan Measles (SM2), an online database system administered by the Ministry of Health, Malaysia to record all activities of investigation and registration of measles cases. All suspected measles cases registered in the e-Notification system (online infectious disease notification system for Ministry of Health, Malaysia) will be auto-entered into the e-Measles system for further investigation and case registration.

### Location and Study Population

This study was conducted in the Selangor State Health Department. Selangor was selected because, compared to other states in Malaysia, Selangor recorded the second highest incidence of measles, 73.1 per million population in 2023.<sup>9</sup> Data collection for this study was carried out from April to May 2024. The study population comprised individuals diagnosed with and reported to have measles in the e-Measles system in Selangor from 2014 to 2023.

### Data Collection and Sampling Method

The inclusion criteria were confirmed measles cases in Selangor, including both Malaysian and non-Malaysian, registered in the e-Measles system from 2014 to 2023. This was based on the Ministry of Health's Case Definitions for Infectious Diseases in Malaysia, which defined a confirmed measles case as a laboratory-confirmed case (presence of measles-specific IgM antibodies or presence of measles virus in clinical samples using culture or molecular techniques) or one that met the clinical case definition (any person with fever and maculopapular and either cough, coryza, or conjunctivitis) epidemiologically linked to a laboratory-confirmed case.<sup>1</sup> Exclusion criteria included vaccine-associated measles (VAM), imported measles cases, and cases with incomplete data specifically pertaining to MMR vaccination status and risk factors. Imported measles cases were excluded from the study to maintain a focus on locally transmitted cases and to accurately assess the determinants of measles cases with complications to generate more relevant and specific insights for local health policy and intervention strategies. The sampling method used in this study was universal sampling of secondary data in the e-Measles system.

### Operational definition

The outcome in this study, which is measles cases with complications, is defined as confirmed measles cases that were registered as any of the following complications in the e-Measles system: diarrhea, pneumonia, otitis media, or subacute sclerosing panencephalitis (SSPE). The independent variables in this study were divided into three main domains: socio-demographic, clinical-related, and vaccine-related factors. The urban area in this study is defined as gazetted areas with their adjoining built-up areas, which had a combined population of 10,000 or more.<sup>10</sup> Apart from that, history of contact with other confirmed measles cases is defined as measles cases with epidemiologically linked with other confirmed measles cases within 21 days before the

onset of rashes.<sup>11</sup> Measles outbreak is defined as the occurrence of two or more laboratory-confirmed cases of measles that are linked either epidemiologically or virologically, with the rash onset dates falling within a range of 7 to 21 days apart.<sup>12</sup> Vaccine-associated measles refers to a condition where a person experiences a rash illness, similar to measles, appearing 7 to 14 days after vaccination with a measles-containing vaccine, but without respiratory symptoms.<sup>34</sup>

### Sample Size

The sample size for this study was determined using the OpenEpi software version 3.01. The calculation took into account the prevalence of measles cases with complications in Nigeria (31%), which was obtained from the study by Onoja et al.<sup>13</sup> To achieve a statistical power of 80% and significance level (alpha) of 0.05, a sample size of 329 was initially calculated. Considering the potential 20% rate of missing data, the minimum required sample size for this study was 395.

### Data Analysis

The collected data were entered into SPSS software version 28.0. Data were summarized using median and interquartile range for continuous variables, and frequency and percentage for categorical variables. Univariate analysis was performed using a simple logistic regression. Only variables with a p-value <0.05 in simple logistic regression were selected for multiple logistic regression (binary) analysis to determine the determinants of measles cases with complications. Differences were considered statistically significant at a p-value of less than 0.05, and adjusted odds ratios (aOR) with 95% confidence intervals (CI) were calculated. All factors underwent an interaction check. Model fit was examined using the Hosmer-Lemeshow goodness of fit, which was considered a good fit with a p-value greater than 0.05. In addition, the Cox and Snell R-squared values were evaluated to further assess model fitness. Multicollinearity among the predictors was also assessed. Finally, the model's predictive accuracy was calculated by determining the percentage of area under the receiver operating characteristic (ROC) curve.

### Ethics Approval

This study was registered in the National Medical Research Registry (Ref. No: NMRR ID-24-00319-TTL). Ethical approval for this study was obtained from the Medical Research Ethics Committee (Ref. No: 24-00319-TTL), and the Faculty Research Ethics Committee (Ref. No: 100 - FPR (PT.9/19) (FERC-EX-24-04)). The Selangor State Health Department granted permission to use data in the e-Measles registry for the purposes of this study. This study adhered to the Malaysian Code of Responsible Conduct in Research to ensure data confidentiality and compliance with ethical standards throughout the research. This study utilized anonymized, de-identified secondary aggregated data to prevent re-identification. This method preserves confidentiality and aligns with ethical research standards, thus eliminating the need for informed consent.

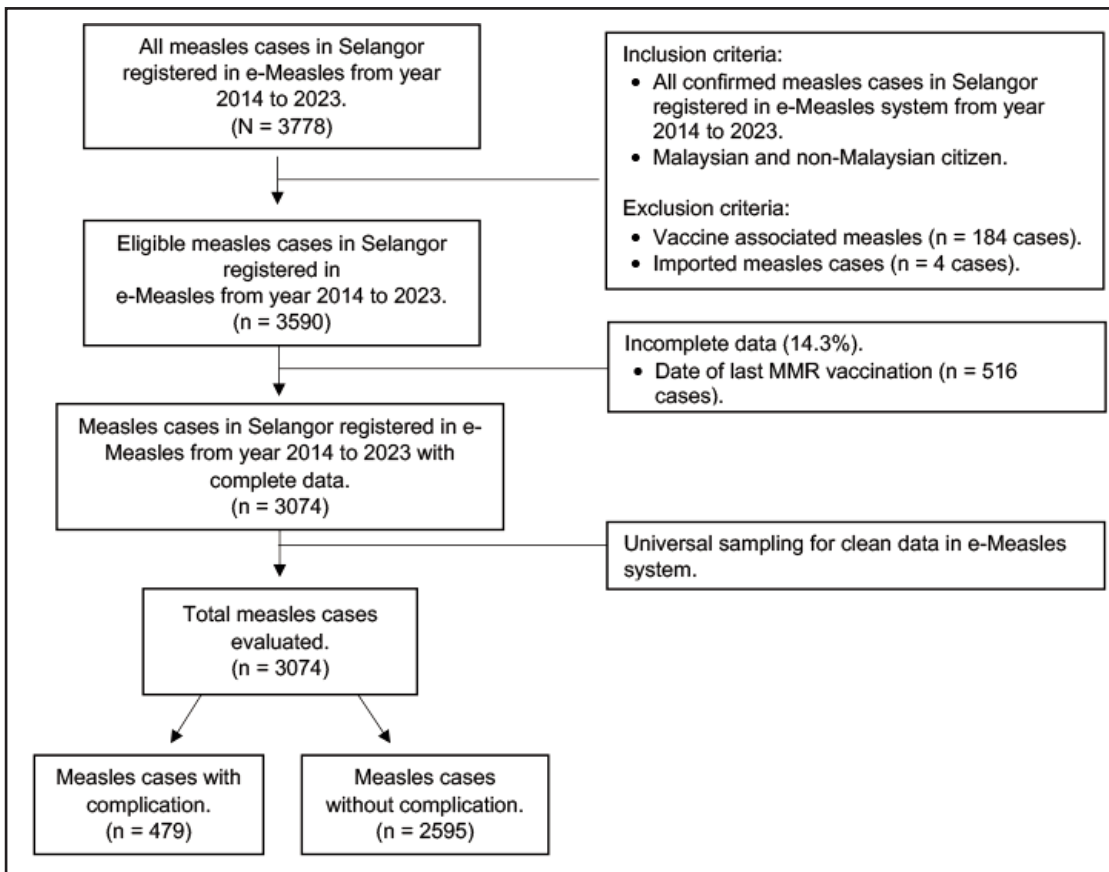


Fig. 1: Flow diagram of data extraction process and sampling.

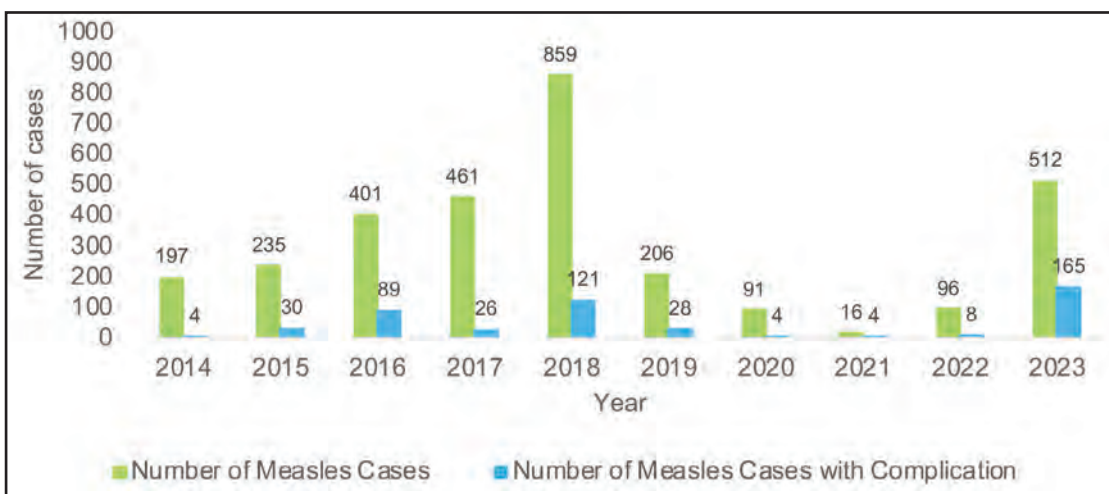


Fig. 2: Trend of measles cases and measles cases with complication in Selangor from year 2014 to 2023.

**RESULTS**

A total of 3778 confirmed measles cases were registered in Selangor from 2014 to 2023. After applying the exclusion criteria (vaccine-associated measles, imported measles cases, and cases with incomplete data), 3074 confirmed measles cases remained and were therefore included in the final analysis of this study (Fig.1). Among the 3074 measles cases, 479 (15.6%) were reported to have complications. The

number of measles cases with complications showed a fluctuating trend from 2014 to 2023, ranging from 4 to 165 cases, with a peak in 2023 (Fig.2). Complications identified were diarrhea (87.6%), pneumonia (11.6%), otitis media (0.6%), and SSPE (0.2%).

Table I shows the sociodemographic characteristics of the measles cases with complications reported in Selangor from

**Table I: Socio-demographic characteristic of measles cases with and without complication in Selangor from year 2014 to 2023 (N = 3074)**

Variables	Total (N = 3074) n (%) <sup>a</sup>	Measles cases with complication (n = 479) n (%) <sup>b</sup>	Measles cases without complication (n = 2595) n (%) <sup>b</sup>
Socio-demographic factor			
Age (in years)			
<1 year old	1294 (42.1)	182 (38.0)	1112 (42.8)
1 to 6 years old	965 (31.4)	141 (29.4)	824 (31.8)
7 to 12 years old	287 (9.3)	54 (11.3)	233 (9.0)
13 to 17 years	93 (3.0)	14 (2.9)	79 (3.0)
≥18 years old	435 (14.2)	88 (18.4)	347 (13.4)
Gender			
Female	1414 (46.0)	209 (43.6)	1205 (46.4)
Male	1660 (54.0)	270 (56.4)	1390 (53.6)
Nationality			
Malaysian	2883 (93.8)	425 (88.7)	2458 (94.7)
Non-Malaysian	191 (6.2)	54 (11.3)	137 (5.3)
Ethnicity			
Chinese	95 (3.1)	15 (3.1)	80 (3.1)
Indian	71 (2.3)	18 (3.8)	53 (2.1)
Malay	2670 (86.9)	380 (79.3)	2290 (88.2)
Others	238 (7.7)	66 (13.8)	172 (6.6)
District			
Sabak Bernam	18 (0.6)	1 (0.2)	17 (0.7)
Gombak	496 (16.1)	11 (2.3)	485 (18.7)
Hulu Langat	772 (25.1)	62 (12.9)	710 (27.4)
Hulu Selangor	73 (2.4)	3 (0.6)	70 (2.7)
Klang	427 (13.9)	158 (33.0)	269 (10.4)
Kuala Langat	170 (5.5)	16 (3.3)	154 (5.9)
Kuala Selangor	75 (2.4)	11 (2.3)	64 (2.5)
Petaling	896 (29.1)	170 (35.5)	726 (28.0)
Sepang	147 (4.8)	47 (9.8)	100 (3.9)
Locality			
Rural	336 (10.9)	31 (6.5)	305 (11.8)
Urban	2738 (89.1)	448 (93.5)	2290 (88.2)

<sup>a</sup>Within total sample.

<sup>b</sup>Within the measles cases with complication or measles cases without complication group.

2014 to 2023. In terms of sociodemographic factors, the majority of measles cases with complications were age < one year old (38.0%), predominantly male (56.4%), Malaysian (88.7%), and Malay (79.3%). Other ethnicities included in this study comprised indigenous people and non-nationals of diverse socio-economic backgrounds who resided in Malaysia for various reasons. Most measles cases with complications were reported in Petaling District (35.5%), and a large proportion of cases were from urban areas (93.5%). Table II shows the clinical-related and vaccine-related characteristics of measles cases with complications reported in Selangor from 2014 to 2023. Regarding clinical background, majority of measles cases with complications fulfilled the measles clinical case definition (76.2%). Most measles cases with complications were sporadic (85.0%), while few cases were reported from measles outbreaks (15.0%). In terms of vaccine-related factors, 81 patients (16.9%) had complete MMR vaccination, 152 (31.7%) had incomplete MMR vaccination, 152 (31.7%) were not eligible for MMR vaccination, and 94 (19.6%) were not vaccinated. The median and interquartile range (IQR) of the duration between the date of the last MMR vaccination and the onset of rash for measles cases with complication was 16.5 months (57.0).

Table III shows the simple and multiple logistic regression analyses of the determinants of measles cases in Selangor from 2014 to 2023. Univariate analysis using simple logistic regression indicated that several factors were associated with complications in measles cases. These included age, nationality, ethnicity, locality, cases that fulfilled the measles clinical case definition, history of contact with other confirmed measles cases, transmission status, hospitalization status, and MMR vaccination status.

After adjusting for confounders using multiple (binary) logistic regression, the final predictors of increased odds of measles cases with complications were non-Malaysian nationality (aOR: 2.10; 95% CI: 1.44, 3.05), urban locality (aOR: 1.84; 95% CI: 1.22, 2.76), cases that fulfilled the measles clinical case definition (aOR: 2.09; 95% CI: 1.59, 2.76), history of contact with other confirmed measles cases (aOR: 4.41; 95% CI: 3.43, 5.67), hospitalized cases (aOR: 5.63; 95% CI: 4.49, 7.07), and cases from measles outbreaks (aOR: 1.55; 95% CI: 1.10, 2.19).

**Table II: Clinical-related and vaccine-related characteristic of measles cases with and without complication in Selangor from year 2014 to 2023 (N = 3074)**

Variables	Total (N = 3074) n (%) <sup>a</sup>	Measles cases with complication (n = 479) n (%) <sup>b</sup>	Measles cases without complication (n = 2595) n (%) <sup>b</sup>
<b>Clinical-related factor</b>			
Fulfil clinical case definition			
No	493 (16.0)	114 (23.8)	379 (14.6)
Yes	2581 (84.0)	365 (76.2)	2216 (85.4)
History of contact with measles cases			
No	2453 (79.8)	276 (57.6)	2177 (83.9)
Yes	621 (20.2)	203 (42.4)	418 (16.1)
Transmission status			
Sporadic	2744 (89.3)	407 (85.0)	2337 (90.1)
Outbreak	330 (10.7)	72 (15.0)	258 (9.9)
Hospitalization status			
No	2218 (72.2)	215 (44.9)	2003 (77.2)
Yes	856 (27.8)	264 (55.1)	592 (22.8)
<b>Vaccine-related factor</b>			
MMR vaccination status			
Complete vaccination	684 (22.3)	81 (16.9)	603 (23.2)
Incomplete vaccination	1073 (34.9)	152 (31.7)	921 (35.5)
Not eligible	955 (31.1)	152 (31.7)	803 (30.9)
Not vaccinated	362 (11.8)	94 (19.6)	268 (10.3)
MMR dose received			
2	684 (22.3)	81 (16.9)	603 (23.2)
1	1073 (34.9)	152 (31.7)	921 (35.5)
0	1317 (42.8)	246 (51.4)	1071 (41.3)
Type of health facility providing MMR vaccination (n = 1757)			
Government	1651 (94.0)	214 (91.8)	1437 (94.3)
Private	106 (6.0)	19 (8.2)	87 (5.7)
Duration between date of last MMR and onset of rash (months) <sup>c</sup>	2.0 (26.0)	16.5 (57.0)	1.0 (21.0)

<sup>a</sup>Within total sample.<sup>b</sup>Within the measles cases with complication or measles cases without complication group.<sup>c</sup>Median (IQR)

## DISCUSSION

The main objective of this study was to determine the determinants of measles cases with complications in Selangor, Malaysia from 2014 to 2023. Based on a representative cross-sectional sample of measles cases in Selangor (N = 3074), significant factors associated with increased odds of measles cases with complications included non-Malaysian nationality, cases from urban localities, cases that fulfilled the measles clinical case definition, cases with a history of contact with other confirmed measles cases, cases from measles outbreaks, and cases that were hospitalized. Age, ethnicity, and MMR vaccination status were identified as statistically significant confounding factors. No significant association was observed between the study outcome with respect to sex, district, number of MMR vaccination doses received, type of health facility providing the MMR vaccination, duration between the date of the last MMR vaccination, and onset of rash.

The higher incidence of complications in 2023 and the lower incidence of complications in 2017, as shown in Figure 2, could be attributed to heightened awareness and surveillance, leading to more thorough reporting of complications, while 2017 might have had underreporting. The increasing trend of measles cases with complications in Selangor post-pandemic COVID-19 was due to measles

outbreaks involving schools and pre-schools in 2023.<sup>14</sup> The possible reason for the occurrence of measles outbreaks is reduced MMR vaccination coverage as well as the high measles R naught of 12 to 18, predisposed to the spread of measles infection.<sup>15</sup> This is similar to a study conducted in Nigeria, which reported that the high frequency of outbreaks in Nigeria contributed to an increased incidence of measles, resulting in a higher risk of measles complications.<sup>16</sup> The majority of measles complications reported in this study were diarrhea followed by pneumonia, otitis media, and SSPE, which is similar to a study conducted in Jerusalem.<sup>17</sup>

In this study, non-Malaysian nationality was significantly associated with measles cases with complications, which could be attributed to the distinct socioeconomic barriers faced by non-Malaysians. These barriers include legal concerns, financial status, language barriers and vaccination status.<sup>18</sup> In addition, measles vaccinations in Malaysia have been provided for free to Malaysian citizens under the National Immunization Program (NIP) since the 1950s.<sup>19</sup> This led to poor measles vaccination coverage among this vulnerable population. The association between non-Malaysian nationality and measles cases with complications can be linked to a complex interplay of socioeconomic factors, and addressing these barriers through targeted interventions, culturally sensitive approaches, and equitable

Table III: Analyses of determinants of measles cases with complication in Selangor from year 2014 to 2023 (N = 3074)

Variables	cOR (95% CI) <sup>a</sup>	p-value <sup>a</sup>	aOR (95% CI) <sup>b</sup>	p-value <sup>b</sup>
<b>Socio-demographic factor</b>				
Age (in years)				
<1 year old	1	ref.	1	ref.
1 to 6 years old	1.04 (0.82, 1.32)	0.714	0.96 (0.73, 1.26)	0.792
7 to 12 years old	1.41 (1.01, 1.97)	0.042 *	1.17 (0.80, 1.73)	0.409
13 to 17 years	1.08 (0.60, 1.95)	0.792	0.79 (0.41, 1.51)	0.482
≥18 years old	1.54 (1.16, 2.05)	0.002 *	1.08 (0.79, 1.49)	0.61
Gender				
Female	1	ref.		
Male	1.12 (0.92, 1.36)	0.258		
Nationality				
Malaysian	1	ref.	1	ref.
Non-Malaysian	2.28 (1.63, 3.17)	<0.001 *	2.10 (1.44, 3.05)	< 0.001 *
Ethnicity				
Chinese	1	ref.	1	ref.
Indian	1.81 (0.84, 3.90)	0.13	1.50 (0.65, 3.44)	0.336
Malay	0.88 (0.50, 1.55)	0.67	0.98 (0.53, 1.79)	0.95
Others	2.04 (1.10, 3.80)	0.024 *	1.95 (0.98, 3.86)	0.054
District				
Sabak Bernam	1	ref.		
Gombak	0.38 (0.04, 3.16)	0.375		
Hulu Langat	1.48 (0.19, 11.34)	0.703		
Hulu Selangor	0.72 (0.07, 7.44)	0.789		
Klang	7.98 (0.91, 75.74)	0.062		
Kuala Langat	1.76 (0.22, 14.15)	0.592		
Kuala Selangor	2.92 (0.35, 24.24)	0.321		
Petaling	3.98 (0.52, 30.11)	0.181		
Sepang	5.99 (0.73, 61.84)	0.074		
Locality				
Rural	1	ref.	1	ref.
Urban	1.92 (1.31, 2.82)	<0.001 *	1.84 (1.22, 2.76)	0.003 *
<b>Clinical-related factor</b>				
Fulfill clinical case definition				
No	1	ref.	1	ref.
Yes	1.82 (1.44, 2.31)	<0.001 *	2.09 (1.59, 2.76)	< 0.001 *
History of contact with measles cases				
No	1	ref.	1	ref.
Yes	3.83 (3.10, 4.72)	<0.001 *	4.41 (3.43, 5.67)	< 0.001 *
Transmission status				
Sporadic	1	ref.	1	ref.
Outbreak	1.62 (1.20, 2.12)	0.001 *	1.55 (1.10, 2.19)	0.011 *
Hospitalization status				
No	1	ref.	1	ref.
Yes	4.15 (3.39, 5.08)	<0.001 *	5.63 (4.49, 7.07)	< 0.001 *
<b>Vaccine-related factor</b>				
MMR vaccination status				
Complete vaccination	1	ref.	1	ref.
Incomplete vaccination	1.22 (0.92, 1.64)	0.162	0.89 (0.63, 1.25)	0.505
Not eligible	1.40 (1.05, 1.88)	0.020 *	1.16 (0.84, 1.61)	0.342
Not vaccinated	2.61 (1.87, 3.63)	<0.001 *	1.40 (0.96, 2.04)	0.073
MMR dose received				
2	1	ref.		
1	1.05 (0.72, 1.54)	0.773		
0	0.63 (0.39, 1.01)	0.059		
Type of health facility providing MMR vaccination				
Government	1	ref.		
Private	1.19 (0.71, 1.97)	0.499		
Duration between date of last MMR and onset of rash (months)				
	1.00 (1.00, 1.05)	0.052		

Note: cOR = crude odds ratio, aOR = adjusted odds ratio, CI = confidence interval, <sup>a</sup>simple logistic regression, <sup>b</sup>multiple logistic regression, \*level of significance at  $\alpha = 0.05$ . None of the 15 interactions were significant. No multicollinearity among the variables was detected in the final model. The Hosmer-Lemeshow goodness-of-fit test indicated a good model fit ( $p = 0.113$ ). Pseudo R-square (Cox and Snell) = 19.4%. The area under the Receiver Operating Characteristic (ROC) curve was 76.9%. No influential outlier (Cook's influential statistics).

healthcare access is crucial to improving measles immunization coverage and reducing the burden of measles among non-Malaysian populations.

Apart from that, the odds of measles cases with complications among cases from urban areas were 1.84 compared to cases from rural areas, and this is similar to the study done by Aworabhi-Okoi et al. (2020), who found that living in urban areas increased the risk of measles infection.<sup>20</sup> The possible reason is reduced MMR vaccine coverage occurred more in urban areas.<sup>21</sup> However, studies on situational analysis of measles cases with complications, especially in urban areas in Malaysia, remain inadequate. Most published studies and situational analyses have focused only on the number of measles cases and their incidence. It is essential to take action to reduce vaccine hesitancy and increase immunization coverage in urban areas. For example, widespread public education campaigns about vaccine safety and effectiveness should be part of strategies, particularly in urban areas where misinformation may be more common.<sup>22</sup>

Cases that fulfilled the measles clinical case definitions (i.e. cases that had a fever, maculopapular rash and one of the '3Cs' (cough, coryza or conjunctivitis) had 2.09 times higher odds of being measles cases with complication. Studies have shown that the clinical case definition has a high predictive value in diagnosing measles; for example, a study determining the measles clinical case definition in New York City reported the negative predictive value of the case definition at 98%.<sup>23</sup> Therefore, Ministry of Health Malaysia agreed on similar clinical case definitions, as most measles cases fulfilled this definition. Other than that, measles cases that fulfil the clinical case definitions need to be investigated thoroughly, as they are more likely to develop measles complications. Additionally, research has shown that cases meeting the clinical case definition for measles are more likely to have an IgM-positive measles virus, highlighting the significance of the clinical case definition in accurately diagnosing measles.<sup>24</sup>

In Selangor, the odds of measles cases with complications were four times if there was a history of contact with other confirmed measles cases, which is similar to a case-control study done in Ethiopia where people who had contact with measles cases within the last 21 days were 3.4 times more likely to get measles complications compared to those who were not in contact with measles cases.<sup>25</sup> Given that close contacts were the most susceptible group, the findings may be explained by the fact that measles is spread via respiratory droplets and the measles infectivity period is four days before and after the onset of rash.<sup>26</sup> This is in support of the public health preventive measures of keeping children home from daycare centers or schools if they are suspected of having measles. Isolation reduces the likelihood of contact and hence the risk of measles transmission. According to the Malaysian government, parents and other caregivers of children suffering from communicable diseases, such as measles, dengue fever, malaria, chickenpox, diphtheria, and hand foot and mouth (HFMD) disease, are eligible for quarantine leave.<sup>27</sup> This particular work leave facilitates isolation while also minimizing the caregiver's burden.

Moreover, this study found that cases of measles outbreaks were significantly associated with measles cases with complications. Previous study done in Mongolia also had shown the significant association between measles outbreaks and measles complications.<sup>28</sup> During measles outbreaks, vaccination status plays a vital role in determining the risk of complications for example, cases that were not vaccinated were much more likely to develop complications from the disease.<sup>29</sup> The measles vaccination might reduce the chance of complications and the severity of the disease, even if it may not provide full protection against infection.<sup>30</sup> Apart from that, measles outbreaks and subsequent complications have been strongly associated with factors such as vaccine hesitancy and low vaccination coverage.<sup>31</sup> Consequently, in Malaysia, the MMR vaccination continues to be an essential intervention for lowering the risk of complications and lessening the impact of measles outbreaks.

In terms of hospitalization status, inpatient cases were significantly associated with measles cases and complications, with an increased odds of 5.63 times compared to outpatient cases. Several studies have highlighted the association between inpatient cases and measles complications compared to outpatient cases. For example, hospitalized patients with measles tend to have more severe cases with complications, as indicated by a study in the United States.<sup>32</sup> Additionally, a study conducted in Italy reported that a significant proportion of hospitalized measles patients developed complications, with pneumonia being a frequent severe complication.<sup>33</sup> In summary, evidence from various studies supported the findings that inpatient cases of measles were significantly associated with a higher risk of complications compared to outpatient cases, indicating the importance of timely and appropriate medical care for measles patients to prevent adverse outcomes.

Awareness campaigns involving the Malaysian Ministry of Health (MOH), political leaders, and non-governmental organizations (NGOs) need to be strengthened, especially for the non-Malaysian population. Furthermore, future studies focusing on the characteristics of this vulnerable population may improve our understanding of the increased risk of measles with complications.

#### STRENGTHS AND LIMITATIONS

The strength of this study is that the confirmatory test for measles was performed at the National Public Health Laboratory (MKAK) Malaysia, which is a WHO-recognized laboratory for measles, thereby reducing interlaboratory bias. Furthermore, this is a population-based study compared with hospital-based studies, which can provide a better picture of this disease in a population. One limitation of this study is that the analysis based on surveillance data is limited by the completeness of the data; for example, insufficient data on parents' socio-demographics such as education level, household income, and the reason the children did not receive their vaccinations is not fully explained in the surveillance system. Apart from that, the analysis of the severity level of the inpatient cases was not performed because the data contained insufficient information. Moreover, although this study controlled for age, ethnicity,

and MMR vaccination status, there remains the potential for residual confounding arising from variables not included in this study.

## CONCLUSION

Measles complications are still prevalent in Selangor and are expected to increase as the incidence of measles continues to increase. Identifying factors that contribute to measles cases with complications helps to comprehend the overall impact of measles on public health and provide insight into the overall burden of the disease in Selangor, Malaysia, as well as help in early detection and management of the disease, leading to better patient outcomes.

## FUNDING

This research did not receive any specific grants from funding agencies in the public, commercial, or not-for-profit sectors.

## ACKNOWLEDGEMENTS

We would like to thank the Director General of Health Malaysia for permission to publish this article. We also express our sincere gratitude to the Selangor Health State Department for granting permission to conduct this study and for the assistance provided by their dedicated health staff during data collection. Additionally, we would also like to extend our appreciation to the Department of Public Health Medicine, Faculty of Medicine, Universiti Teknologi MARA (UiTM), for their valuable support throughout the study.

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# Procedural safety and outcome of rigid bronchoscopy in malignant central airway obstruction

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## ABSTRACT

**Introduction:** Malignant central airway obstruction (CAO) affects 20-30% of patients with primary lung cancer and is associated with poor prognosis without treatment. Therapeutic rigid bronchoscopy (RB) is an essential diagnostic and therapeutic tool in this group of patients. We aimed to assess the overall procedural outcomes and safety of RB for symptomatic malignant CAO at our institution.

**Materials and Methods:** Retrospective chart review included 57 patients with malignant CAO who underwent RB between March 2016 and June 2022.

**Results:** Our cohort comprised 75.4% males with a median age of 60 years. Isolated right and left main bronchus involvement was observed in 50% and 41.7% of the patients, respectively, with 21.1% experiencing concurrent tracheal involvement. In our cohort, 80% had primary lung cancer, with 35.1% of these cases being squamous cell carcinoma. The re-canalization success rate was 98%, with no intraoperative or immediate postoperative mortality. More than half of the patients (54.4%) required airway stenting to maintain patency after re-canalization. Patients with tracheal involvement, non-intrinsic type of CAO, and right main bronchus involvement had a higher likelihood of requiring airway stenting. There were 24 cases with mild, self-limiting complications and 3 cases with severe procedure-related complications. The 30-day survival rate of our cohort was 91.6%.

**Conclusion:** RB is a safe and effective procedure in patients with malignant CAO. Long term prospective data from multiple centers in our region are anticipated to determine long-term safety and outcomes.

## KEYWORDS:

*Rigid bronchoscopy, malignant central airway obstruction*

## INTRODUCTION

Central airway obstruction (CAO) is defined as symptomatic airway obstruction with an endoluminal diameter of <50%, affecting the trachea, right or left main bronchus, or bronchus intermedius, as observed on imaging or bronchoscopy<sup>1</sup>. Malignancy is one of the most common causes of lung cancer globally<sup>1,2</sup>, often from primary lung cancer or lung metastases<sup>3</sup>.

In Malaysia, lung cancer is the most prevalent type of cancer and most patients are diagnosed at an advanced stage.<sup>4</sup> Hence, most patients with malignant CAO present life-threatening symptoms and are often ineligible for definitive treatment. Around 20-30% of primary lung cancer cases have malignant CAO at diagnosis,<sup>1</sup> and approximately 5% develop it during treatment.<sup>2</sup> This complication worsens the already dismal prognosis of lung cancer patients.<sup>4,5</sup>

Nowadays, interventional bronchoscopists typically classify malignant CAO into three categories: extraluminal (extrinsic), endoluminal (intrinsic), or mixed.<sup>6</sup> This classification is key for determining the bronchoscopic management. The EpiGETIF registry demonstrated that most malignant CAOs are intrinsic (43.3%) or mixed types (41.5%), as bronchogenic tumors are the primary cause.<sup>7</sup>

Rigid bronchoscopy (RB) is a minimally invasive technique within the realm of interventional pulmonology that allows effective restoration of airway patency in patients with symptomatic CAO. Surgery is often contraindicated, and systemic chemotherapy and radiotherapy may not provide immediate relief.<sup>8,9</sup> Therefore, RB is a crucial bridging therapy.

While RB for malignant CAO is well established in developed countries, data on its efficacy and safety in developing countries are limited. Thus, we conducted a retrospective observational study in a single institution in Malaysia to assess the demographic characteristics, overall procedural outcomes, and safety of RB in symptomatic malignant CAO.

## MATERIALS AND METHODS

### Study Design & Setting

This was a retrospective chart review of consecutive adult patients undergoing RB for malignant CAO from March 2016 to June 2022 at Sarawak General Hospital, Malaysia. The study protocol was approved by the Medical Research and Ethics Committee of the Ministry of Health Malaysia (NMRR-ID-23-00354-T5Q (IIR)). The requirement for informed consent was waived owing to the retrospective nature of the study.

### Rigid Bronchoscopy Procedure for Malignant CAO

#### i. Pre-procedural Planning

All patients underwent contrast-enhanced chest CT to

This article was accepted: 28 May 2025

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evaluate airway invasion and pulmonary vascular integrity. The decision to proceed with airway intervention was tailored to each patient's fitness, imaging results, and, crucially, the multidisciplinary tumor board's assessment of treatment options and disease prognosis.

### Rigid Bronchoscopy Tumour Debulking

All patients scheduled for RB were first referred to an anesthesiologist for risk assessment and optimization, as most procedures were performed in emergency or semi-emergency situations in the operating theatre. At our institution, most cases were performed under total intravenous anesthesia (TIVA), with assisted ventilation, intermittent neuromuscular blockade, and invasive monitoring, including arterial blood pressure.

Rigid tracheoscopes and bronchoscopes of various sizes (*Efer-Dumon*<sup>®</sup>, *Efer Endoscopy, La Ciotat, France*) were used at our institution. In most cases, a flexible bronchoscopy system (*BF-1TH190, BF-H190, Olympus Medical, Japan*) was used in conjunction with a rigid bronchoscopy system to allow for more flexibility and reach.

The tumor-debulking modalities employed in our institution primarily comprise mechanical methods and thermal devices. Mechanical coring was performed using rigid tracheal or bronchial tubes and dilatation using various sizes of controlled radial expansion (CRE) balloon catheters (*CRE*<sup>®</sup>, *Boston Scientific, Natick, MA*). Thermal devices included an electrocautery snare or probe and argon plasma coagulation (APC) (*VIO 300D, Erbe Elektromedizin, Tübingen, Germany*), as well as cryo-technology (*ERBECRYO*<sup>®2</sup>, *Erbe Elektromedizin, Tübingen, Germany*) with at reusable 1.9-mm/2.4-mm cryoprobe or single use 1.1-mm/1.7-mm/2.4-mm flexible cryoprobe.

After the procedure, patients were monitored in the ICU or HDU for 24-48 hours. Chest radiography was performed to confirm lung re-expansion and identify any complications. An oncology referral was performed before discharge to ensure the timely initiation of treatment. The first surveillance bronchoscopy was typically scheduled 4-6 weeks post-RB.

### Airway Stenting

Airway stenting was considered when airway patency remained at <50% after debulking and dilatation. The bronchoscopist chose between metallic and silicone stents based on the airway's geometry and orientation. At our institution, partially covered or uncovered self-expanding metallic tracheobronchial stents (*SEMS, UltraflexTM 94, Boston Scientific, Natick, MA*) of various sizes were available. For silicone stents, the straight and Y-bifurcation stents (*DumonTM, Novatech, La Ciotat, France*) were available.

SEMS placement was performed under direct bronchoscopic vision, sometimes under fluoroscopy and guidewire guidance. Silicone stents were deployed using a dedicated stent loader and applicator, with the pulling-back technique being the most commonly used. On-site customization of the silicone stent is sometimes necessary to accommodate a patient's specific airway geometry.

### Definition

The primary outcome was defined as successful airway recanalization, with  $\geq 50\%$  patency achieved by eye balling<sup>10</sup>. As a secondary outcome, we assessed the proportion of patients who were able to undergo oncology treatment, inferred from their fitness for subsequent surveillance bronchoscopy.

Bleeding is classified according to standard definition<sup>11</sup> as follows:

- Grade 1: Bleeding is self-limiting and resolves with suctioning for <1 minute.
- Grade 2: Bleeding requires suctioning for >1 minute or instillation of a hemostatic agent.
- Grade 3: Bleeding necessitates balloon blockade for <20 minutes or premature termination of the procedure.
- Grade 4: Bleeding requires balloon blockade for >20 minutes, transfusion, admission to the ICU, resuscitation, or bronchial artery embolization.

### Statistical Analysis

SPSS (*version 20; Chicago, IL, USA*) was used for the data analysis. Normality was assessed using the Shapiro-Wilk test. The results are presented as mean  $\pm$  standard deviation (SD) for normally distributed variables and as median and interquartile range (IQR) for non-normally distributed variables. Categorical data were expressed as absolute numbers and percentages, and compared using Pearson's chi-square test or Fisher's exact test. Independent sample t-tests and Mann-Whitney tests were used to compare normally and non-normally distributed variables, respectively, between the groups. A p-value of <0.05 was considered significant.

## RESULTS

A total of 61 RB procedures were performed during the study period, of which 57 (93.4%) were for indications of malignant CAO and were included in this study.

### Baseline demographic and obstruction characteristic

Our cohort comprised 75.4% male patients with an overall median age of 60.0 (IQR 54.0-68.5) years. No ethnic differences were observed.

Isolated unilateral main bronchi involvement was the most common, affecting RMB in 50.0% and LMB in 41.7% respectively. Out of these cases, trachea was concurrently involved in 21.1%. Concurrent segmental airway involvement was reported in 56.1% of the cases. The predominant obstructions were purely intrinsic (45.6%) and mixed (36.8%), while 17.5% were purely extrinsic.

The most common histological finding was primary lung cancer (of 45/57, 78.9%), with nearly one-third (35.1%) being squamous cell carcinoma. Secondary metastatic malignancy was observed in 21.1% (12/57) of the patients. The complete details can be found in Table I.

### Overall procedure characteristic and outcome

All RB procedures were performed in the operation theatre under TIVA with assisted ventilation and intermittent

Table I: Baseline demographic, obstruction and procedural characteristic

Clinical demographic and obstruction characteristic	
<b>Median age (IQR), years</b>	<b>60.0 (54.0-68.5)</b>
Gender, n (%)	
Male	43 (75.4)
Female	14 (24.6)
Race, n (%)	
Native Sarawak	21 (36.8)
Chinese	20 (35.1)
Malay	16 (28.1)
Obstruction site, n (%)	
Trachea	12 (21.1)
Right main bronchus	25 (43.9)
Bronchus intermedius	8 (14.0)
Left main bronchus	23 (40.4)
Segmental airway involvement, n (%)	32 (56.1)
Obstruction type, n (%)	
Pure Extrinsic	10 (17.5)
Pure Intrinsic	26 (45.6)
Mixed	21 (36.8)
Procedural characteristic and outcome	
Median procedural duration (IQR), minutes	90 (60-135)
Debulking modalities, n (%)	
Rigid forceps	40 (70.2)
Electrocautery	37 (64.9)
Cryoprobe	31 (54.4)
Argon plasma coagulation	18 (31.6)
Mechanical balloon dilatation	12 (21.1)
Recanalization success, n (%)	56 (98.0)
Patient requiring stent, n (%)	31 (54.4)
Stents, n (%)	
Self expanding metallic stent	15 (48.4)
Silicone stent	16 (51.6)
Patient fit for subsequent surveillance bronchoscopy, n (%)	33 (57.9)
<b>Final histology</b>	<b>N (%)</b>
Primary lung malignancy	45 (78.9)
Squamous cell carcinoma	20 (35.1)
Adenocarcinoma	11 (19.3)
Small cell carcinoma	8 (14.0)
Carcinoid	1 (1.8)
Adenoid cystic carcinoma	1 (1.8)
Mucoepidermoid carcinoma	1 (1.8)
Combined squamous and small cell carcinoma	1 (1.8)
Non-small cell pleomorphic carcinoma	1 (1.8)
Combined small and large cell neuroendocrine carcinoma	1 (1.8)
Secondary metastatic lung malignancy	12 (21.1)
Metastatic colorectal adenocarcinoma	3 (5.3)
Metastatic oesophageal carcinoma	2 (3.5)
Hodgkin's lymphoma	2 (3.5)
Metastatic breast adenocarcinoma	1 (1.8)
Metastatic thyroid adenocarcinoma	1 (1.8)
Metastatic ovarian adenocarcinoma	1 (1.8)
Metastatic renal adenocarcinoma	1 (1.8)
Metastatic pleomorphic liposacorma	1 (1.8)

neuromuscular blockade. The median procedural duration was 90 (IQR 60-135) minutes.

The debulking modalities in our cohort included energy devices, cryotechnology, and mechanical methods. Energy devices comprising electrocautery and APC were used in 64.9% and 54.4% of the cases, respectively. Cryoprobes was used for cryo-recanalization and cryo-extraction in 54.4% of cases. Mechanical recanalization involved rigid forceps (70.2%) and, a CRE balloon (21.1%) coupled with manual coring via the rigid tracheal or bronchial tube (Table II).

Primary recanalization was successful in 98% (56/57) of patients, with one unsuccessful attempt. Overall, 57.9% (33/57) of patients received systemic oncological therapy and underwent follow-up surveillance bronchoscopy. As shown in Table II, there were no significant differences in fitness for surveillance bronchoscopy based on malignancy type, sex, tracheal or main bronchi involvement, or airway stenting.

Among 32 patients with complete follow-up data, the 30-day survival rate was 91.6%. No intra-operative or short-term procedure-related mortality was observed in this cohort. Mild

**Table II: Factors associated with fitness for surveillance bronchoscopy post rigid bronchoscopy**

Factors		Fit, n (%)	p-value
Malignancy	Primary	26 (57.8)	0.972
	Metastatic	7 (58.3)	
Gender	Male	24 (55.8)	0.577
	Female	9 (64.3)	
Types of CAO	Intrinsic	12 (46.2)	0.115
	Non-intrinsic	21 (67.7)	
Trachea involvement	Involved	9 (75.0)	0.177
	Spared	24 (53.3)	
Left main bronchus involvement	Involved	16 (69.6)	0.142
	Spared	17 (50.0)	
Right main bronchus involvement	Involved	13 (52.0)	0.426
	Spared	20 (62.5)	
Bronchus intermedius involvement	Involved	3 (37.5)	0.208
	Spared	30 (61.2)	
Segmental airway involvement	Involved	20 (62.5)	0.426
	Spared	13 (52.0)	
Airway stent	Stented	21 (67.7)	0.100
	No stent	12 (46.2)	

**Table III: Factors associated with likelihood of airway stenting**

Factors		Stent, n (%)	p-value
Malignancy	Primary	27 (60.0)	0.099
	Metastatic	4 (33.3)	
Gender	Male	26 (60.5)	0.106
	Female	5 (35.7)	
Types of CAO	Intrinsic	0 (0.0)	<0.001
	Non-intrinsic	31 (100.0)	
Trachea involvement	Involved	11 (91.7)	0.004
	Spared	20 (44.4)	
Left main bronchus involvement	Involved	12 (52.2)	0.783
	Spared	19 (55.9)	
Right main bronchus involvement	Involved	19 (76.0)	0.004
	Spared	12 (37.5)	
Bronchus intermedius involvement	Involved	5 (62.5)	0.619
	Spared	26 (53.1)	
Segmental airway involvement	Involved	14 (43.8)	0.068
	Spared	17 (68.0)	

bleeding (Grade 1-2) was occurred in 42% (24/57) of the patients and was self-limiting. However, severe complications occurred in three patients (5.3%): one with grade 4 bleeding leading to premature procedure termination, another with a partial mucosal tear at the main bronchus requiring stenting, and one who developed post-procedure pneumothorax requiring intercostal chest tube drainage.

#### **Factors associated with likelihood of airway stenting**

Approximately 54.4% (31/57) required airway stenting to maintain the recanalization effect during the same RB procedure, with more silicone stents (51.6%) placed than SEMs (48.4%).

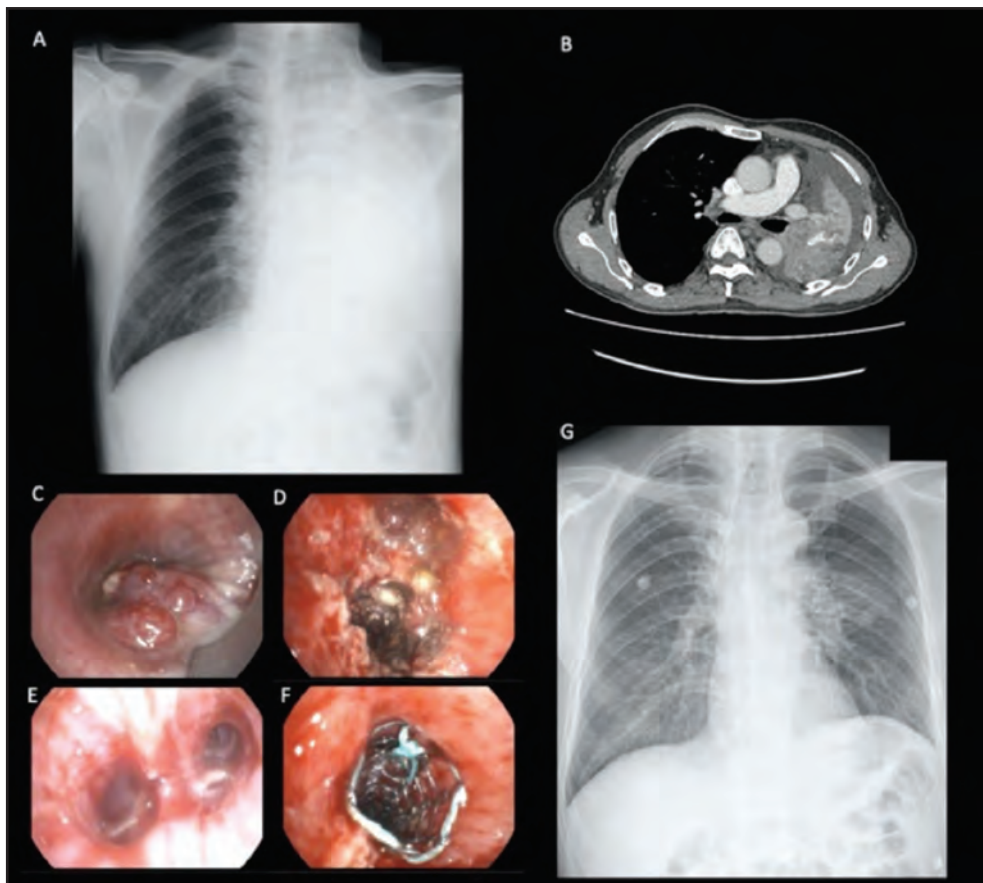
Among patients with tracheal involvement, 91.7% (11/12) required stenting. SEMs were placed in three patients, while Y-bifurcation silicone stents were placed in eight patients due to concurrent main bronchi involvement (Figure 2.0). In contrast, only 44.4% (20/45) of patients with main bronchi obstruction without tracheal involvement required stenting, with 12 SEMs and 8 straight silicone stents deployed (Figure 1.0).

Tracheal involvement significantly increased the need for stenting to maintain patency (91.7% vs. 44.4%;  $p < 0.01$ ). None of the patients with pure intrinsic CAO required a stent, whereas all patients with pure extrinsic or mixed types of CAO required stenting ( $p < 0.001$ ). Nonintrinsic CAO ( $p < 0.001$ ) and right main bronchus involvement ( $p < 0.05$ ) were associated with a higher likelihood of airway stenting in our cohort. (Table III)

For patients with airway stents, the overall stent-related complication rate was 35.5% (11/31), which included stent migration ( $n=4$ ) and restenosis due to tumor ingrowth ( $n=7$ ).

#### **DISCUSSION**

Our study demonstrated that RB is effective and safe for malignant CAO in our region. The primary success rate, defined as successful recanalization, was 98%, with 5.3% severe adverse events. This aligns with the literature, where the recanalization rate was 93.7-100%, and almost half of the patients reported symptomatic improvement in breathlessness and quality of life<sup>12-14</sup>. The EpiGETIF registry



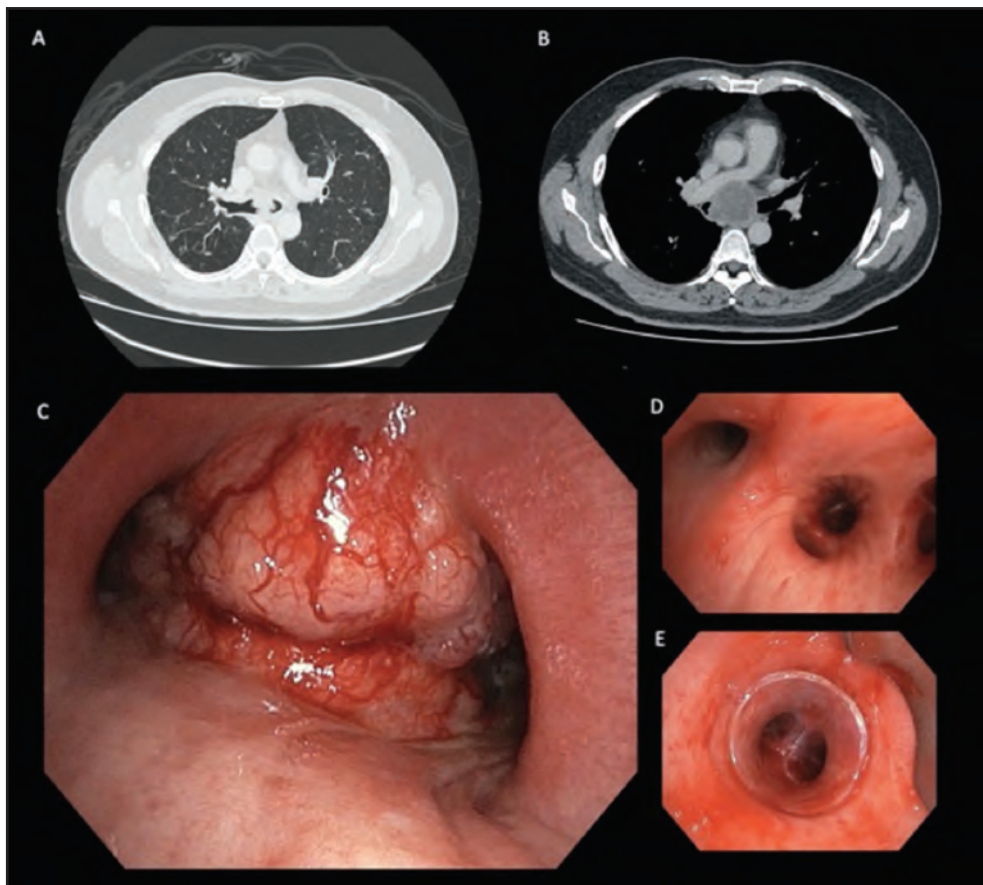
**Fig. 1:** Representative Case (1) – Small cell carcinoma causing left main bronchus malignant CAO. Patient presented with constitutional symptoms and exertional dyspnoea, chest radiograph on arrival shown complete left lung collapse (Panel A). Contrast enhanced CT thorax demonstrated a proximal hilar tumour eroding into the left main bronchus (LMB) with preserved pulmonary vasculature associated with distal atelectasis (Panel B). Rigid bronchoscope revealed a mixed type CAO in the LMB (Panel C) which was then debulked with hot and cold modalities to reveal the left upper and lower lobe bronchi (Panel D). The basal segment of left lower lobe was patent (Panel E) and a partially covered self-expanding metallic stent was deployed in the LMB across the LUL bronchus. Patient then underwent chemotherapy (carboplatin and etoposide) and remained well eight months later with left lung aeration (Panel G).

reported that 87.8% of patients achieved a mean improvement of 4.1 points in the Borg dyspnea score<sup>7</sup>. By contrast, Akash et al.<sup>13</sup> reported that 8.3% of severe adverse events required escalation of care, whereas Kim et al.<sup>14</sup> found a 17% rate of immediate post-procedural complications. Furthermore, the AQUIRE Registry reported an overall complication rate of 3.9% across 15 centers, ranging from 0.9% to 11.7%, whereas the EpiGETIF registry showed an overall complication rate of 10.9%<sup>7,15</sup>. Moreover, nearly two-thirds (57.9%) of our patients received systemic oncological therapy following RB intervention. Malignant CAO severely affect the survival of patients with lung cancer, with a median survival of 2-3 months<sup>16</sup>. Previous studies indicate that therapeutic bronchoscopy for CAO can yield median survival rates comparable to those without CAO (8.4 vs. 8.2 months)<sup>17</sup>. Hence, patients eligible for airway interventions should always be considered for RB when resources are permitted.

The median age and sex distribution of patients with malignant CAO in our cohort were in concordance with regional data from Asia and France, where the median age was 63 years and two-thirds of the patients were male<sup>7,13,14,18</sup>.

The finding that primary lung cancer was the leading cause of malignant CAO was also consistent with cohorts from Asia, France, and the UK<sup>1,7,13,14</sup>. This is an important observation, as NSCLC in our region is often linked to EGFR mutations, which respond well to oral tyrosine kinase inhibitors<sup>19</sup>. Therefore, efforts to relieve malignant CAO are essential to ensure that patients receive effective targeted treatment. In our cohort, adenocarcinoma was a minor cause of malignant CAO (19.3%) compared with other tumor types. Therefore, chemotherapy and immunotherapy are the preferred treatment options for patients with a performance status (PS) <2. Interestingly, most CAO types in our cohort were extrinsic and mixed, likely due to the predominance of NSCLC or SCLC in our cohort, which causes direct airway invasion or compression from lymphadenopathy, rather than tumors predominantly in the airway, such as carcinoid and salivary gland tumors<sup>20,21</sup>.

Multiple techniques were employed for airway recanalization. Rigid forceps and mechanical coring using a rigid tube were the most commonly used in our cohort. While effective in removing tumor tissue, these methods can be challenging when the axis of the airway cannot be



**Fig. 2:** Representative Case (2) - Metastatic squamous cell carcinoma with main carinal infiltration causing central airway obstruction. Patient presented to us with hemoptysis and exertional dyspnea with CECT Thorax showing intraluminal mass over the main carinal region obstructing bilateral main bronchi (Panel A) and a large subcarinal lymph node (Panel B). Under rigid bronchoscopy, main carinal infiltration was confirmed with a highly vascular tumour over the main carina causing a mixed type CAO (Panel C). Cautious bronchoscopic examination confirmed distal airway patency bilaterally (Panel D). A silicone Y-stent was deployed over the main carina which translated to immediate relief of breathlessness. Patient then went on to palliative chemotherapy.

confidently aligned with the rigid bevel or in angulated regions of the lung<sup>6,22</sup>. Hence, various flexible ancillary tools are used in tandem with flexible bronchoscopy, including an electrocautery probe/snare, APC, and cryotherapy. Combining flexible and rigid bronchoscopy harnesses the strengths of both systems, offering greater access to the airway while maintaining flexibility for interventions at acute angles.

In our cohort, 54.4% of patients required a stent to maintain airway patency after debulking. The factors associated with an increased likelihood of stenting included tracheal involvement, RMB involvement, and extrinsic CAO type. Notably, two of the (21.1%) patients presented with tracheal involvement. In these patients, either side of the main bronchi was invariably be involved, and we showed that the likelihood of stent insertion was significantly higher than those without tracheal involvement. This is consistent with the findings from other Asian centers. A Japanese cohort reported that 54% of stents were placed in the trachea and carina, while a Singaporean cohort found that 52% of CAO involving the trachea required stenting<sup>8,13</sup>. Akash et al.<sup>13</sup> noted a CAO incidence of 33.3% at the LMB compared to 29% at the RMB and BI, resulting in a higher stent requirement rate of 67% for the LMB. Conversely, the

Japanese cohort demonstrated a higher airway stenting rate in the RMB and BI (23%) than in the LMB (22%)<sup>8</sup>. Additionally, the Canadian and UK cohorts found a significantly higher CAO incidence in the RMB and BI (64.8% and 63%, respectively) than in the LMB (29.7% and 22%, respectively)<sup>1,12</sup>. The higher incidence of CAO in the RMB may be related to its anatomical characteristics – wider, shorter, and more aligned with the trachea, making it susceptible to the deposition of inhaled carcinogens. Additionally, increased airflow distribution to the right lung and the right lymphatic drainage system may also be contributing factors<sup>23,24</sup>, a hypothesis supported by extrapolating from our understanding that pulmonary tuberculosis is more common in the right lung<sup>25</sup>. Lastly, we have shown that in patients with pure extrinsic type of malignant CAO, a stent is invariably needed, in line with the literature<sup>6</sup>. Only around 4 in 10 patients with mixed-type CAO required airway stenting post intervention, as the debulking process itself likely addressed the intrinsic portion of the CAO, rendering stenting unnecessary.

We also reported a significant stent-related complication rate of 35.5%, primarily due to stent migration and restenosis due to tumor ingrowth. This rate exceeds the literature rate of 16.6% for stent migration and 42% for stent related infections

in the AQuIRE Registry<sup>15</sup> and the 0.7% complication rate in the EpiGETIF registry.<sup>7</sup> We attribute this to the limited stent options in our region to match the airway configuration in malignant CAO, which is often distorted and not in standard anatomical alignment. Although silicone stents allow customization<sup>26</sup>, the pressure points may not be ideal, leading to granulation tissue formation or migration. Customized three-dimensional printed stents based on individual airway anatomy may offer a future solution for this issue<sup>27</sup>.

Our study has several limitations. Its retrospective design and single-institution data limit its ability to draw firm conclusions. However, it provides valuable insights into CAO demographics in our region and represents the largest cohort in Malaysia to date, which will be useful for clinicians. While we reported low adverse events in the intraprocedural and immediate postprocedural periods, long-term data on survival, quality of life, and exercise tolerance are lacking. Additionally, performance status or the Borg Score Scale would be a better tool for evaluating the outcomes. However, we did not have access to this information owing to the retrospective nature of the study. Nonetheless, in 32 patients with full follow-up, the 30-day survival rate was 91.6%, similar to the 30-day mortality rate of 14.8% in the AQuIRE registry. Finally, further research is needed to determine the methods for monitoring and preventing stent-related complications.

## CONCLUSION

Rigid bronchoscopy is a safe and effective treatment for malignant CAO in patients with advanced malignancy. We achieved a recanalization success rate of 98%, with 54.4% of patients requiring airway stenting. Tracheal and right main bronchus involvement as well as non-intrinsic CAO were more likely to require stenting. Future multicenter studies in this region are expected to provide long-term safety and outcome data.

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# Accuracy of age estimation method by panoramic radiographic examination in paediatric dental patients: A diagnostic study in Indonesia

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## ABSTRACT

**Introduction:** Cases of violence and criminality in children continue to increase, with implications for the increasing need for identification measures, especially for living victims for judicial or law enforcement purposes. Teeth are one of the indicators that determine a child's identity. The methods often used to estimate a child's age through dental radiographic examination during the period of deciduous and mixed teeth are the AI Qahtani, Demirjian, and Blenkin & Evans methods.

**Materials and Methods:** An analytical cross-sectional study was conducted. The children's ages were estimated using dental examination and 103 digital panoramic radiographs. Descriptive statistics, normality test followed by difference test based on five parameters: p-value Wilcoxon test, Intraclass Correlation Coefficient (ICC), Spearman correlation coefficient, and Bland-Altman analysis by looking at % out of Limit of Agreement and looking at Mean Difference.

**Results:** Wilcoxon's p-value showed that Blenkin's method and Demirjian's method vs. chronological age were significantly different, whereas AI Qahtani's method was not significantly different. The Demirjian method had the highest ICC. Spearman's correlation coefficient was strongly correlated between Blenkin's and Demirjian's methods and the chronological age ( $r \geq 0.8$ ). The Bland-Altman analysis showed that the Demirjian Method had the best % out of LOA = 5.8, while the AI Qahtani Method had the most minor differences.

**Conclusion:** The Demirjian method showed results closest to the patient's chronological age.

## KEYWORDS:

Children, chronological age, crime and violence, estimation method

## INTRODUCTION

Recently, news on crimes against children has become rampant. Almost every day, there is a lot of news in the media and perceptions from the community that reports

incidents of violence, kidnapping, abuse, neglect of children, and others. According to the World Health Organization (WHO), violence against children is an act of mistreatment or mistreatment of children in the form of physical, emotional, or sexual harm; neglect of care; and exploitation for commercial interests that are real or not, which can endanger their health, survival, dignity, or development. The potential for violence against children includes physical, emotional, sexual, and economic violence. In 2023, the Ministry of Communication and Information, along with the Indonesian Child Protection Commission, stated that Indonesia was in a child abuse emergency.<sup>1,2</sup>

Age identification is widely used in paediatric dentistry, forensics, anthropology, archaeology, and orthodontics. Age determination in live child individuals is generally used for various purposes such as 1) identification of unknown individuals at the scene of a crime or accident; 2) estimating the chronological age of children with missing or doubtful birth certificates; 3) falsification of working age, marriage age, and athlete's age; 4) guardianship of children; 5) immigration; 6) rape; and 7) legal evidence to determine whether a person is legally a child or an adult, where there are different legal and judicial processes for children and adults.<sup>3,4</sup> Treatment planning and prognosis of a particular orthodontic treatment depends mainly on the age of the teeth. Chronological age (CA) can also be used as an essential indicator to determine abnormal eruption sequences to implement preventive measures.<sup>5,6</sup>

Forensic dentistry is a branch that uses teeth for identification and judicial or law enforcement. Teeth are used as a medium of identification because they are the hardest part of the body and are resistant to degradation, decomposition, chemical influences, and high-temperature influences. Dental maturity is considered a more accurate indicator of biological maturity in children, because it is least affected by nutritional and endocrine status. A widely researched method for estimating dental age is to analyze tooth growth on radiographs. This method was chosen because it is easy to perform and non-invasive, especially in estimating the age of living individuals.<sup>7-10</sup> Various methods are currently used to predict age, such as skeletal maturation, physical examination using anthropometric measurements, and

This article was accepted: 04 April 2025

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dental examination. Human skeletal growth and maturation are unique to each individual, but dental examination techniques are considered the best in assessing true chronological age, especially during the age range when teeth are undergoing morphological development.<sup>11-13</sup> It is known that different genetic and environmental factors may cause individuals of the same chronological age to have other body characteristics and developmental levels. Therefore, before using an age estimation method for legal purposes in any population, it is essential to validate the accuracy and applicability of the technique in a specific population.<sup>14,15</sup>

To the best of our knowledge, no clinical study has officially stated the most appropriate age estimation method for child identification in Indonesia. As long as we are in the field of forensic odontology, there is no consensus among forensic odontology practitioners on the dominant age estimation method for Indonesian children. Previous studies have only compared several methods with their respective advantages and disadvantages; therefore, there is no answer in cases that require age estimation in Indonesia. Some adopted methods used for age estimation through radiographic examination of children's teeth in the deciduous and mixed phases include the Al Qahtani, Demirjian, and Blenkin and Evans methods. The author hopes to answer the question of using one of the most appropriate age estimation methods for Indonesian children. By knowing the most suitable method and achieving high accuracy, the success of the work carried out can be achieved optimally. Therefore, this study aimed to evaluate the accuracy and applicability of three established dental age estimation methods in an Indonesian paediatric population.

## MATERIALS AND METHODS

This study was approved by the Ethical Committee of Health Research, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia (046/S3/KEPK/FKG/7/2024).

### Study Design

An analytical study with a cross-sectional design was conducted between October and December 2024. The study was designed to estimate the age of paediatric patients through a dental examination on digital panoramic radiographs by comparing the measurement results using the Al Qahtani, Demirjian, and Blenkin & Evans methods to the patient's chronological age. Panoramic radiographs were obtained from paediatric patients at the Radiology Installation of the Dental and Oral Hospital (RSGM), Faculty of Dentistry, Trisakti University.

### Sample Size and Data Collection

Based on our calculations, the minimum sample size for this study was 73. The panoramic radiographs examined were panoramic radiographs of paediatric dental patients who came to the RSGM Faculty of Dentistry, Trisakti University, from September 2023 to September 2024.

The study inclusion criteria were panoramic images of healthy individuals, no systemic disease, children aged 0-16 years, no orthodontic treatment, no bilateral anomalies or

pathology in the maxillofacial region, history of trauma, and no bilateral permanent tooth loss, caries, restoration, root canal treatment, or apical pathology. The exclusion criteria were as follows. incomplete tooth seeds in region 3 and poor quality panoramic radiography results.

A competent forensic odontologist performed the measurements using these three methods. All measurement results were recorded and matched with the patients' chronological age according to the hospital's Medical Record data.

## Procedures of Age Estimation<sup>8,10</sup>

### Demirjian Method

First, the gender of the subjects was identified. The method relies on a panoramic radiograph of the left mandibular permanent teeth, from the central incisor to the second molar. Next, we assessed the tooth maturation stages of each tooth to convert them into the Demirjian maturation score according to the sex-specific table. The scores were then calculated to obtain the Total Demirjian Maturation Score, which was converted according to the Demirjian age estimation table.

### Blenkin Evans Method

First, the gender of the subjects was identified. The method relies on a panoramic radiograph of the left mandibular permanent teeth, from the central incisor to the second molar. We then assessed the tooth maturation stages of each tooth, similar to the Demirjian Classification of maturity, to convert it into the Blenkin maturation score according to the sex-specific table. Next, we calculated the scores to obtain the Total Maturation Score and converted the score according to the age estimation table.

### Al-Qahtani Method

This method uses atlas and presents reference images for each age group from birth to 23 years. First, the tooth development stages include crown formation, tooth positioning in relation to the alveolar bone, root formation, and apical closure of each tooth on one side of both jaws. Then, we matched the development stages assessed on panoramic radiographs with the Atlas. Next, we determined the age range that represents the best match between the radiograph and the Atlas.

### Statistical Analysis

Data cleaning and tabulation were performed using Microsoft Excel, which was then exported to the Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, version 25.0). Descriptive statistics were used to display the characteristics of the study participants, followed by a normality test. T-tests were conducted to compare the measurement results of the three methods on the CA of patients, based on five parameters: Wilcoxon Signed-Rank Test, Intraclass Correlation Coefficient (ICC), Spearman correlation coefficient, and Bland-Altman analysis by looking at % out of Limit of Agreement (% out of LOA) and looking at mean difference.

A Wilcoxon test was conducted to determine whether there was a significant difference between chronological age and

the measurement results of the age estimation method. The ICC is a reliability test between two observations: CA-Blenkin, CA-Demirjian, and CA-Al Qahtani. Spearman's Correlation was then used to determine whether there was a linear relationship between the variables CA-Blenkin, CA-Demirjian, and CA-Al Qahtani. The % of LOA was used to calculate the percentage of extreme measurement results from the total sample.

## RESULTS

A total of 103 digital panoramic radiographs of pediatric patients were obtained at an oral hospital. Table I presents the range (minimum and maximum), standard deviation of chronological age, and the three methods measured.

Table II shows the results of the age difference test on CA against the other three age estimation methods using non-parametric tests, namely the Wilcoxon signed-rank paired test and the Spearman correlation coefficient. Other tests are ICC (Intraclass Correlation Coefficient), Bland-Altman (% of the Limit of Agreement), and mean differences.

Wilcoxon's p-value shows that Blenkin's Method and Demirjian's method vs. CA are significantly different, meaning that the median ages in Blenkin's method and Demirjian's method median vs. CA are significantly different. However, in Al-Qahtani, the median age was not significantly different from the median CA. The ICC test showed that all three methods were either very good or excellent. Excellent when ICC was  $> 0.75$ . (Ref Christine Konieczka et al). However, the Demirjian method had the highest ICC value (ICC = 0.8).

The Spearman correlation coefficient strongly correlated with the Blenkin and Demirjian methods for chronological age ( $r \geq 0.8$ ). The Bland-Altman analysis shows that all methods have a percentage of the Limit of Agreement below 10%, and the Demirjian Method has the smallest percentage LoA; therefore, it can be said to be better. Bland-Altman analysis shows a gray belt, which means that the observation points (one point is one sample) are within the tolerance limit. This belt was formed based on data variation; the more varied it is, the wider it will be. The minimum to maximum range is wider, meaning that it is more varied. Bland-Altman analysis assesses the range of the upper and lower limits. What is essential in Bland Altman's analysis is the percentage of observations outside the tolerance limit, which is called the %out of the Limit of Agreement (%out of LoA).

Analysis using Mean Differences shows that the Al Qahtani Method has the smallest mean difference value (closest to chronological age) compared to the other two methods. Demirjian showed the highest ICC. ICC = reliable when  $\geq 0.75$ , and in Al Qahtani or Demirjian all above 0.75. Regarding the size of the numbers, it cannot be compared; the important thing is that the interpretation if above 0.75 is said to be reliable.

## DISCUSSION

Age determination is a major concern in both medical and legal procedures. Dental age identification is an important

procedure in clinical science, forensics, and legal procedures. As we know, cases of crimes and violence against children tend to increase annually. Therefore, indirectly, the need for age estimation through dental examinations of children for law enforcement purposes has also increased, especially in living individuals. In this situation, the role of pedodontists and forensic odontologists becomes essential, and, of course, adequate knowledge is needed. This study was conducted in Jakarta, the largest metropolitan city in Indonesia with the highest population heterogeneity. Many people from various regions and almost all ethnic groups live in Jakarta.<sup>16,17</sup>

The radiographic method is simple, rapid, economical, and minimally invasive for age identification. It can be used as a diagnostic tool to estimate the age of the deceased or living subjects in all communities. However, radiographic examination still has some ethical considerations regarding the side effects of X-rays.<sup>8,9,18,19</sup> This study used existing digital panoramic radiographs of the patients, which were only used in this study. It did not perform Direct radiographic photographs of the patients were not obtained. Panoramic radiographs were obtained from patients treated at the study hospital. This was chosen to minimize exposure to the subjects.

Teeth are an essential parameter that can be used for age estimation. This is because teeth have morphological characteristics that differ with age due to processes such as enamel and dentin calcification, crown development, and root development until they are fully formed. Forensic odontologists play an essential role in determining the unknown fetal age in cases of premature birth and abortion. Primary tooth mineralization starts at 6 to 7 weeks intrauterine, and with the help of histological methods, tooth mineralization can be observed up to 12 weeks before it is detected by radiographs.<sup>8,9</sup>

The t-test of this study shows that the Blenkin-Evans method does not have the best value for all parameters used. Although they met the standard criteria for these parameters, they did not achieve the best value. Each dental age estimation method is developed based on individuals from different ethnic backgrounds. The Blenkin-Evans Method was adapted from the Demirjian Method to suit the Australian pediatric population.<sup>10,11,20,21</sup> However, research conducted by Sahin in the pediatric population of Turkey (2025) showed that the Blenkin-Evans method gave the closest results to chronological age compared to other methods. Different genetic and environmental factors can cause individuals with the same CA to have different body characteristics and developmental levels.<sup>22,24</sup>

Of the five parameters of this study, the Al-Qahtani Method has the best value in 2 parameters. Al-Qahtani explained the development of teeth and their eruption rate in one region in the upper jaw and one area in the lower jaw in each individual with an age range of 6-23 years. The obstacle to the Al-Qahtani method is that the Al-Qahtani method diagram is only based on the development of the second and third molar teeth, which can affect the age results of the study due to the different developmental states of the third molar teeth. Al Qahtani et al. published a new dental-age assessment method in 2010. The London Atlas is an

illustrated textbook that assesses each tooth's formation and eruption stages and then matches it to one of 31 age-category illustrations representing tooth formation and eruption. The London Atlas was initially tested on British and Bangladeshi ethnic subjects and was shown to be more precise than the Schour, Massler, and Ubelaker methods, and potentially more accurate than Demirjian calculations.<sup>5,23</sup>

This study indicates that the Demirjian method has the best value among the three parameters. This shows that the age estimation results of the Demirjian Method are the closest to the actual chronological age of the patient. The advantages of the Demirjian Method include detailed calculation of the maturity level of each tooth and the conversion of the number from the Demirjian maturation score, which leads to a number that will be converted to the age from the Demirjian Table. The Demirjian Method is much more complex and detailed than other age estimation methods that use the atlas of dental development stages.<sup>24-26</sup>

The results of this study are also in line with research by Kotecha, who found that the most widely accepted system is the system developed by Demirjian, which assesses children's dental development individually. The stages of the left mandibular tooth formation (excluding the third molars) were evaluated. Individual scores for each of the seven stages were summed, and these were converted into a single dental age representing the average age of the child with that score. However, research conducted by Pliska et al. in 2024 showed that the Willems Method was more accurate than the Demirjian Method. In this case, the estimation results of the Demirjian method exceed the chronological age. Based on these points, it can be said that the Demirjian Method is not highly correlated when used in different populations.<sup>3,5,24</sup> This study was conducted in Jakarta, the capital city with the highest heterogeneity in Indonesia, but it does not rule out the possibility that there are tribes or ethnic groups that do not live in Jakarta, but in other regions within Indonesia.

## CONCLUSION

After using the Al-Qahtani, Demirjian, and Blenkin methods and performing statistical analyses, the study concluded that the Demirjian Method measurement results are closest to the chronological age of children in the Indonesian child population.

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# Echocardiography detection rate of newly diagnosed valvular heart disease amongst patients 12 years old and beyond, referred for transthoracic echocardiography in tertiary care settings

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## ABSTRACT

**Introduction:** Valvular heart disease (VHD) is a significant contributor to cardiovascular morbidity and mortality. Despite global awareness, data on the epidemiology and clinical profile of VHD in Malaysia remain limited. This study aimed to determine the echocardiographic detection rate of newly diagnosed VHD in tertiary care centres in Perak, Malaysia, as well as to examine its clinical characteristics, aetiologies, severity, associated comorbidities, and the rate of patient acceptance for valve intervention.

**Materials and Methods:** A retrospective, multi-center study was conducted involving patients who underwent echocardiography over a six-month period. The data were then analysed to address the study objectives. Statistical analysis was performed using SPSS version 25.0.

**Results:** Of the 12,610 patients who underwent echocardiography, 946 were newly diagnosed with VHD, yielding a detection rate of 7.5%. The median age of VHD patients was 67 years, with a slight female predominance. The median left ventricular ejection fraction (LVEF) was 57%. Approximately 66% of patients were symptomatic, with dyspnoea being the most common symptom (46.85%). Pulmonary hypertension was observed in 35.3% of patients. Atrial fibrillation (AF) was present in 12.2% of cases. Mitral regurgitation (MR) was the most common valve lesion (34.1%), followed by tricuspid regurgitation (TR, 32.3%) and aortic regurgitation (AR, 19.2%). Functional aetiology was the most frequent cause of VHD, accounting for 59%. Rheumatic heart disease (RHD) was responsible for 51.7% of mitral stenosis (MS) cases, while degenerative causes predominated in AR (74.9%), aortic stenosis (AS) (84.6%), and primary MR (71.4%). The majority of VHD cases were mild (65.6%), with 29.2% moderate and 5.1% severe. Among VHD patients, 34.7% had clinically significant moderate or severe disease. In this subgroup, the predominant aetiologies were functional for MR (52.1%), TR (96.7%), and pulmonary regurgitation (PR, 100%), while degenerative

causes were prevalent in AR (63.6%) and AS (84.3%), and RHD was the leading cause of MS (57.1%). Multiple valve involvement was observed in 72.6% of cases. Two-valve involvement accounted for 52.5%, with MR and TR being the most common combination (68.7%), mainly due to functional causes. AS was significantly associated with hypertension and dyslipidaemia. MR showed strong associations with AF, chronic kidney disease (CKD), and ischemic heart disease (IHD), while TR was more common in females. Despite 6.7% of patients requiring valve intervention, only 24.2% consented. A total of 36.4% declined intervention, primarily due to concerns about advanced age (62.5%) and fear of surgery (29.2%), while 39.4% remained undecided.

**Conclusion:** The echocardiographic detection rate of newly diagnosed VHD was 7.5%, with functional MR being the most common lesion (20.7%). The majority of patients refused valve intervention, primarily due to old age. This study provides valuable insight into the burden and management challenges of VHD in Malaysia.

## KEYWORDS:

*Newly diagnosed, valvular heart disease, echocardiography, detection rate, aetiology, severity, Malaysia*

## INTRODUCTION

Valvular heart disease (VHD) is a major cause of cardiovascular mortality and morbidity worldwide, affecting more than 2% of the global population.<sup>1</sup> This could potentially be the 'new cardiac epidemic' and remains a crucial clinical burden.<sup>2,3</sup> Based on data from large population-based epidemiological studies in the USA, the estimated prevalence of significant VHD in developed countries is 2.5%.<sup>2</sup>

Although mild to moderate VHD mostly does not require intervention, severe VHD is associated with impaired quality of life and prolonged hospitalization and can progress to

This article was accepted: 28 May 2025

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complication such as heart failure, arrhythmia and death. There is no effective medical therapy, but early detection of individuals with moderate to severe VHD who may benefit from valve intervention can reduce or eliminate symptoms, prevent further complications and improve outcome of morbidity and mortality.<sup>4</sup>

Following adequate clinical evaluation, echocardiography is the key technique used to confirm the diagnosis of VHD and to assess its severity. With advancement in catheter-based valvular interventions, outcomes of VHD patients have improved dramatically, particularly for those at high risk for surgical interventions. In fact, recent updates in both the American College of Cardiology (ACC) and European Society of Cardiology (ESC) clinical practice guidelines reflect the change in management of these novel techniques, with stronger recommendations for adopting such treatment options.<sup>5,6</sup> Large datasets provide real-world insights into the epidemiology of VHD, and better use of analytics may lead to improvements in care.<sup>7</sup>

Despite the clinical significance of VHD, there is a lack of epidemiological data describing its burden in Malaysia.<sup>3</sup> Thus, the primary objective of this study was to describe the echocardiography detection rate of VHD in four main tertiary centres in Perak over six-month period. Our secondary objectives were to explore the baseline characteristics, severity and aetiology of VHD, to test for association between comorbidities and types of VHD and finally, to explore the proportion of patients who are agreeable for cardiac intervention and reasons for refusing such intervention

## MATERIALS AND METHODS

### Study design and Study Population

This was a retrospective study conducted in Perak's four main tertiary healthcare centres: Hospital Raja Permaisuri Bainun, Hospital Taiping, Hospital Teluk Intan, and Hospital Slim River over a period of six months (July 2023 - December 2023). All patients who undergone outpatient and inpatient echocardiography examinations during the study period will be included. Health records will then be reviewed for those patients with newly diagnosed VHD and data will be collected.

### Inclusion criteria

All patients aged 12 years and above at the time of diagnosis who undergone echocardiography from July 2023 until December 2023 at the healthcare centres mentioned in the study design were included in this study.

### Exclusion criteria

The exclusion criteria included pre-existing VHD, non-valvular cardiac structural abnormalities, trivial regurgitations, prosthetic heart valves and repeated echocardiography.

### Echocardiography procedures

Echocardiography is the first-line modality for assessing of valvular regurgitation and stenosis.<sup>3</sup> All transthoracic echocardiography were performed by experience cardiac sonographers who were trained to assess VHD. All study sites used the American Society of Echocardiography (ASE)

guidelines and standards to define VHD and as the reference parameters to classify severity of VHD.<sup>8-10</sup> The aetiology of the VHD were then determined based on the ACC/AHA and ESC/EACTS Guidelines for the management of valvular heart disease.<sup>5-6</sup>

### Data collection

Case Report Forms (CRFs) were filled out by designated personnel in the respective hospitals if patients met the inclusion and exclusion criteria. All information for the CRFs, particularly demographics, comorbidities, clinical symptoms, echocardiography findings, aetiology, treatment strategy, and medications, was extracted from the medical records and echocardiography reports and images. This study utilized a paper CRF to collect the required variables/data. The study investigator entered relevant data from patients into the study CRF.

### Statistical Analysis

All statistical analyses were performed by using Statistical Package for Social Science (SPSS) software version 25.0. The normality of data distribution was assessed using the Kolmogorov-Smirnov test. Categorical variables were presented in frequency and percentage (%). Numerical variables were expressed in median with interquartile range (IQR) as our data showed a non - normal distribution.

The number of patients with echocardiographic evidence of VHD relative to the total number of patients who underwent echocardiography during the study period will be recorded to establish the echocardiography detection rate of VHD. Those with newly diagnosed VHD were classified according to demographic, clinical symptoms, echocardiographic findings and aetiology of VHD. Pearson chi-square and Fisher's exact test were used to look for any association between comorbidities and types of VHD among those patients with newly diagnosed VHD (Table IV).

## RESULTS

### Echocardiography detection rate of newly diagnosed VHD

Of all 12,610 patients who underwent echocardiography over a 6 month period, 946 were newly diagnosed with VHD, corresponding to a echocardiography detection rate of VHD of 7.5%

### Baseline Demographics among patients with newly diagnosed valvular heart disease (Table I)

The median age was 67 year-old (IQR 57, 74). There was a slight female preponderance, with 51.7% female and 48.3% male patients. Division according to ethnicity shows that Malays were the majority (53.8%), followed by Chinese (31.5%), Indians (13.6%), and other ethnicities (1.1%). 27.2% of patients were current or ex-smokers, while 72.8% were non-smokers. 66% of patients were symptomatic. The most common symptom was dyspnoea (46.5%), with most patients classified as NYHA Class II (49.7%). Other symptoms included chest pain (17.4%), leg swelling (16.2%), orthopnoea (11.3%), and paroxysmal nocturnal dyspnoea (9.7%). The median left ventricular ejection fraction (LVEF) was 57% (IQR 42, 63). Pulmonary hypertension was present in 35.3% of patients with VHD. The median pulmonary arterial systolic pressure was 33 mmHg (IQR 25, 44).

Table I: Baseline demographics among patients who has newly diagnosed valvular heart disease

	n (%)
Age (Range) (years) <sup>a</sup>	67 (57, 74)
BMI (kg/m <sup>2</sup> ) <sup>a</sup>	25.4 (22.7, 28)
Ethnicity	
Malay	509(53.8)
Chinese	298(31.5)
Indian	129(13.6)
Others	10(1.1)
Gender	
Male	457(48.3)
Female	489(51.7)
Smoking status	
Current/Ex-smoker	257(27.2)
Non-smoker	689(72.8)
Alcohol consumption	
Current or Ex drinker	22
Non-alcohol consumer	924(97.7)
Family history of valve disease	
Yes	16(1.7)
No	930(98.3)
Comorbidities	
Yes	870 (92)
No	76 (8)
Types of Comorbidities	
Diabetes Mellitus	388(41.0)
Dyslipidaemia	144(15.2)
Hypertension	709(74.9)
IHD	204(21.6)
Kidney disease	156(16.5)
Stroke	44(4.7)
Peripheral vascular disease	3(0.3)
Clinical symptoms	
Symptomatic	624 (66)
Asymptomatic	322 (34)
Types of Symptoms	
Dyspnoea	440 (46.5)
Chest pain	165 (17.4)
Leg swelling	153 (16.2)
Orthopnea	107 (11.3)
Paroxysmal nocturnal dyspnoea	92 (9.7)
Palpitation	59 (6.2)
Syncope	11 (1.2)
NYHA Classification for Dyspnoea	
NYHA Class I	406 (42.9)
NYHA class II	470 (49.7)
NYHA class III	70 (7.4)
NYHA class IV	0 (0)
ECG Rhythms	
Sinus rhythm	808 (85.4)
Atrial Fibrillation	115 (12.2)
Atrial Flutter	4 (0.4)
Atrial tachycardia	1 (0.1)
Bradyarrhythmia	18 (1.9)
Medications	
Antiplatelet	443 (46.8)
Statins	676(71.5)
Angiotensin Converting Enzyme inhibitor (ACE-I)/	
Angiotensin Receptor Blockers (ARB)/ Angiotensin Receptor	
Nepriylsin Inhibitor (ARNI)	421 (44.5)
Beta blockers	465 (49.2)
Diuretics	265 (28)
Digoxin	1
Warfarin	26 (2.7)
Non Vitamin K Antagonist Oral anticoagulation	44 (4.7)
Mineralocorticoid Receptor Antagonist (MRA)	95(10)
Sodium Glucose Transport Protein 2 inhibitors (SGLT2-I)	56 (5.9)

<sup>a</sup>Median (IQR)

**Table II: Aetiology of valvular heart disease among patients who has newly diagnosed valvular heart disease**

Etiology	AR	AS	PR	PS	TR	MR	MS
	n =354	n =104	n = 132	n = 2	n = 595	n = 628	n = 29
Degenerative (%)	75.98	84.62	0	0	0	21.98	48.3
Rheumatic (%)	10.17	12.5	0	0	0.17	7.8	51.7
Infective Endocarditis (%)	0.85	0	0	0	0.51	0.32	0
Congenital (%)	1.13	0.96	0	100	0.17	0	0
Inflammatory (%)	0.28	0	0.76	0	0.84	0.64	0
Ischemic (%)	0	0	0	0	0	8.28	0
Functional (%)	0	0	99.24	0	98.15	60.67	0
Aortic root disease (%)	11.58	1.92	0	0	0	0	0
Hereditary (%)	0	0	0	0	0	0.32	0

**Table III: Valve Lesion severity for overall or Division by Type of valve lesion among patients who has newly diagnosed valvular heart disease**

	Overall	MR	MS	TR	AR	AS	PR	PS
Severity	n=1844	n= 628	n= 29	n=595	n=354	n=104	n=132	n=2
Mild (%)	65.7	66.4	56.6	59.5	72.3	51.0	87.0	50
Moderate (%)	29.2	29.9	36.7	33.5	26.0	30.8	13.0	0
Severe (%)	5.10	3.7	6.7	7.0	1.7	18.2	0	50

**Table IV: Association between comorbidities and types of VHD**

Types of VHD		HTN	DM	Dyslipidemia	IHD	Kidney disease	Stroke	Peripheral vascular disease
MR	n(%)	526 (83.8)	290 (46.2)	97(15.5)	162(25.8)	130(20.7)	33(5.3)	1(0.2)
(n=628)	p-value	0.134 <sup>a</sup>	0.298 <sup>a</sup>	0.102 <sup>a</sup>	0.019 <sup>a</sup>	0.001 <sup>a</sup>	0.753 <sup>a</sup>	0.180 <sup>b</sup>
MS	n (%)	22(75.9)	13(44.8)	9(31.0)	6(20.7)	4(13.8)	0	0
(n=29)	p-value	0.836 <sup>a</sup>	0.793 <sup>a</sup>	0.035 <sup>b</sup>	0.832 <sup>a</sup>	0.805 <sup>b</sup>	0.393 <sup>b</sup>	1.000 <sup>b</sup>
TR	n(%)	517(86.9)	281(47.2)	98(16.5)	145(24.4)	120(20.2)	28(4.7)	2(0.3)
n=595	p-value	0.881 <sup>a</sup>	0.719 <sup>a</sup>	0.143 <sup>a</sup>	0.475 <sup>a</sup>	0.232 <sup>a</sup>	0.150 <sup>a</sup>	1.000 <sup>b</sup>
AR	n (%)	270(76.3)	121(34.2)	59(16.7)	80(22.6)	60(17.0)	21(5.9)	1(0.3)
n=354	p-value	0.794 <sup>a</sup>	0.000 <sup>a</sup>	0.400 <sup>a</sup>	0.648 <sup>a</sup>	0.862 <sup>a</sup>	0.166 <sup>a</sup>	1.000 <sup>b</sup>
AS	n(%)	89(85.6)	47(45.2)	26(25.0)	18(17.3)	10(9.6)	3(2.9)	0
n=104	p-value	0.014 <sup>a</sup>	0.408 <sup>a</sup>	0.004 <sup>a</sup>	0.243 <sup>a</sup>	0.041 <sup>a</sup>	0.466 <sup>b</sup>	1.000 <sup>b</sup>
PR	n (%)	126(95.5)	72(54.5)	28(21.2)	42(31.8)	32(24.2)	3(2.3)	1(0.8)
n=132	p-value	0.478 <sup>a</sup>	0.858 <sup>a</sup>	0.696 <sup>a</sup>	0.337 <sup>a</sup>	0.431 <sup>a</sup>	0.044 <sup>a</sup>	0.455 <sup>b</sup>
PS	n(%)	1(50.0)	1(50.0)	0	0	0	0	0
n=2	p-value	0.438 <sup>b</sup>	1.000 <sup>b</sup>	1.000 <sup>b</sup>	1.000 <sup>b</sup>	1.000 <sup>b</sup>	1.000 <sup>b</sup>	1.000 <sup>b</sup>

<sup>a</sup>Pearson's Chi Square, <sup>b</sup>Fisher's exact

**Table V: Decision for Valve Intervention among patients who has newly diagnosed valvular heart disease**

Decision for Valve intervention	n (%)
Agreed	16 (24.2%)
Undecided	26 (39.4%)
Refused	24 (36.4%)
Reason for Refusal	n (%)
Old age	15 (62.5%)
Fear of surgical risk	7 (29.2%)
Severe comorbidities	1 (4.2%)
Symptoms tolerable	1 (4.2%)

**Types, aetiology and severity of VHD (Table II & III)**

Mitral regurgitation (MR) was the most frequent valve lesion (34.1%), followed by tricuspid regurgitation (TR) (32.3%), aortic regurgitation (AR) (19.2%), pulmonary regurgitation (PR) (7.2%), aortic stenosis (AS) (5.6%), mitral stenosis (MS) (1.6%), and pulmonary stenosis (PS) (0.05%). The most common aetiology for overall valvular heart disease (VHD) was functional, accounting for 59%. Rheumatic heart disease (RHD) accounted for 51.7% of MS, while degenerative causes

represented 74.9% of AR and 84.6% of AS. Functional MR was the most common aetiology for all cases of MR, and degenerative aetiology was the predominant cause of primary MR (71.4%). In terms of severity, there were 65.6% mild, 29.2% moderate, and 5.1% severe valve lesions. MR was the most common valve lesion for both mild and severe severity, at 34.4% and 24.4%, respectively, whereas TR was the most common valve lesion for moderate severity at 34.9%.

### Moderate to severe subgroup

Moderate or severe VHD comprises 34.7% of all VHD cases. Among those with moderate or severe VHD, the most common aetiology was functional for MR (52.6%), RHD for MS (61.5%), functional for TR (97.9%) and PR (100%); and degenerative for AR (63.6%) and AS (84.3%).

### Rheumatic Heart Disease

RHD constitute 6.2% of all VHD, affecting the mitral valve in 56.1% (43% MR, 13.1% MS) and aortic valve in 43% (31.6% AR, 11.4% AS). 52.6% of RHD are of moderate or severe severity.

### Multiple Valves

Multiple valves were involved in 72.6% of cases. Two-valve involvement accounted for 52.5%, with MR plus TR being the most common combination (68.7%). The most common aetiology for two-valve involvement were functional MR and functional TR. 33.8% of patients had three valves involved, with MR plus TR plus AR being the most common combination (46.6%). The most common aetiologies for three-valve involvement were degenerative AR, functional MR, and functional TR. Four valves were involved in 12.7% of cases, with MR plus TR plus AR plus PR being the most common combination at 66.7%. The most common aetiology for four-valve involvement were degenerative AR, functional MR, functional TR, and functional PR.

### Comorbidities and associations (Table IV)

AF was strongly associated with MR ( $p < 0.001$ ) and TR ( $p < 0.001$ ). It was present in 12.2% of VHD cases, with MR and TR each comprising 34% ( $p < 0.001$ ). AS was strongly associated with hypertension ( $p=0.014$ ) and dyslipidaemia ( $p=0.004$ ). Hypertension was present in 84.8% of AS cases, while dyslipidaemia was present in 24.8% of AS cases. MR had a strong association with AF ( $p=0.001$ ), Chronic kidney Disease (CKD) ( $p=0.0001$ ), and ischaemic heart disease (IHD) ( $p=0.019$ ), with MR being the most common valve lesion in AF (34.3%), CKD (36.4%), and IHD (35.8%).

### Classification by LVEF

23.7% of our population had a LVEF of  $\leq 40\%$ , 11.3% had a LVEF between 41% and 49%, and 64.6% had a LVEF of  $\geq 50\%$ . The highest proportion of LVEF  $\leq 40\%$  was in the MR group ( $p < 0.001$ ). In our study, most patients with LVEF  $\leq 40\%$  were on beta blockers (70%) and ACE-I/ARB/ARNI (59%). However, only a small percentage of patients were on MRA (27.2%) and SGLT2-I (18%).

### Valvular Intervention (Table V)

6.7% of patients required valve intervention, but only 24.2% consented. Meanwhile, 36.4% declined, and 39.4% were uncertain ( $p < 0.001$ ). The primary reasons for refusal included old age (62.5%), fear of surgical risks (29.2%), and severe comorbidities (4.2%) ( $p < 0.001$ ).

## DISCUSSION

This is the first echocardiography-based multicenter study to determine the detection rate of VHD in Malaysia. The overall detection rate of 7.5% among patients referred for transthoracic echocardiography suggests a substantial burden of previously undiagnosed VHD in the tertiary care

setting. Compared to Manjunath et al., who reported a detection rate of 9.7% in a similar echocardiography based study in India,<sup>11</sup> the slightly lower rate in our study may reflect differences in RHD prevalence as India has a higher prevalence of RHD.<sup>12</sup>

Globally, the most common valve pathology is RHD affecting the mitral valve. In developed countries, however, degenerative disease is more prevalent, paralleling the increasing proportion of the aging population.<sup>5,13-15</sup>

As regards to the distribution of VHD in our study, MR was the most frequent valve lesion (34.1%). The most common aetiology was functional MR (60.67% of all MR). The most common cause of primary MR was degenerative (22% of all MR). Rheumatic MR accounted for only 6.2% of all VHD. Valve degeneration was the predominant aetiology for AR and AS.

This contrasts with the study by Manjunath et al, whereby Rheumatic MR was the most common primary valve lesion. That study indicated that calcific degeneration was the main aetiology of AS whereas rheumatic aetiology dominated the AR cases.<sup>11</sup>

Our results show some similarity to the Euro Heart Survey, another hospital based multicentre study of VHD involving 5001 patients from 92 centres in 25 European countries. In that study, the most common lesions in were AS and MR, mainly attributed to degenerative causes. The mean age in that study was  $65 \pm 14$  years with 83.2% population are  $\geq 50$  years.<sup>16</sup>

Another large Swedish nationwide hospital-based register study showed the most common lesions were AS followed by MR, also mainly due to degenerative causes. The majority of VHD were diagnosed in the elderly (68.9% in subjects aged  $\geq 65$  years).<sup>17</sup>

Both the Euroheart study and the Swedish study above were conducted in European countries which were developed nations, which consist of more elderly population.<sup>16,17</sup> The similarity in the predominant aetiology of VHD in those studies to our study could be explained by the increase in elderly population in Malaysia. Based on findings of the department of statistics Malaysia, the old population (65 years and over) increased from 2.5 million (7.6%) to 2.6 million (7.7%) in the fourth quarter of 2024 as compared to the fourth quadrant of 2023.<sup>18</sup> The decrement in rheumatic heart disease cases in our study could be due to better accessibility to health care and antibiotic therapy.

There were no population based studies on the incidence or prevalence of RHD in adult patients in Malaysia.<sup>19</sup> The only relevant study focused on patients undergoing mitral valve repair at a tertiary heart centre, revealing that RHD accounted for 46.7% of all mitral valve repair.<sup>20</sup>

In contrast to our study which consist mainly of adult population, the worldwide age-standardized incidence rate (ASIR) was very much higher in children less than 15 years of age at 52.3 cases per 100,000 children in 2019.<sup>21</sup> In Sabah, Malaysia whereby RHD is endemic, the average incidence of pediatric RHD from 2015 until 2018 was 2.19 per 100000

population.<sup>22</sup> The most common VHD in this study was MR followed by AR.<sup>22</sup>

Although women represent a larger share of VHD cases globally, they have frequently been underrepresented in the key studies that inform treatment guidelines and have been shown to experience worse postoperative outcomes.<sup>23</sup> In our study, females make up a substantial portion, at 51.7%.

Our study identified several significant associations between VHD and baseline characteristics or comorbidities. The first association was found between AF and both MR and TR. Previous studies have established AF as a risk factor for developing MR and TR.<sup>24-27</sup> Our study also showed that AS was strongly associated with hypertension and dyslipidemia. The CANHEART Aortic Stenosis Study showed that both hypertension and dyslipidemia have independent and dose-response associations with incident AS.<sup>28</sup> Additionally, another study indicated that hypercholesterolemia and hypertension are significantly associated with severe symptomatic AS.<sup>29</sup> There was also a significant association between female gender and TR, similar to two earlier studies that reported a higher prevalence of TR in women.<sup>30,31</sup> Lastly, our study established a significant association between MR with CKD and IHD. CKD patients developed MR due to either degenerative mitral annular calcification or functional.<sup>32-33</sup> Ischemic MR occurs due to left ventricular dysfunction in IHD.<sup>34-36</sup>

Heart failure with reduced EF (HFrEF) is defined as heart failure with an LVEF of  $\leq 40\%$  while Heart failure with mildly reduced EF (HFmrEF) is defined as heart failure with an LVEF of 41% to 49%.<sup>37</sup> Recent studies revealed that VHD contributes to 5.0%–13.6% of HFrEF and HFmrEF cases.<sup>38,39</sup> The most recent heart failure guidelines strongly recommended for guideline directed medical therapy for HFrEF.<sup>40</sup> In our study, the use of ACE inhibitors, ARBs, ARNI, and beta blockers among patients was commendable and consistent with these guidelines. However, the adoption of SGLT2 inhibitors and MRAs remains low, highlighting the need for increased awareness regarding these treatments.

In our study, valve intervention was not performed in 75.8% patients despite having adequate indications for intervention. The main reasons were patient indecision and refusal due to old age. This contrasts with the EURO HEART study, where valve intervention was not performed in 31.8% of eligible patients. The primary reasons for not proceeding with valve interventions were symptoms regression under medical treatment and old age.<sup>16</sup> Our study allows us to identify the reasons behind patients refusal of valve intervention and provide insights on how to address this issue during future discussions with patients.

Therefore, the findings of our study suggests that there is a need for policy makers in Malaysia to formulate policies that focus on the management of VHD especially degenerative type valvular heart disease. This includes provision of specialist training to increase the number of cardiothoracic surgeons and cardiologist with expertise in the management of valvular heart disease in Malaysia.

## LIMITATIONS

Since our study is not a population-based study, it is subjected to inherent biases. Symptomatic lesions may be overrepresented due to the nature of research conducted in hospital settings. Furthermore, patients with severe lesions may be more inclined to seek treatment at these facilities, which could skew the observed patterns of severity.

Despite the efforts of the investigators, accurately assigning etiologies to all VHD cases based on echocardiographic findings remains challenging and may not be as reliable as data obtained from surgical or autopsy studies.

## CONFLICTS OF INTEREST

All authors of this study have no conflicts of interest to declare.

## ETHICAL APPROVAL

The ethical approval was obtained from Medical Research & Ethics Committee's (MREC), Ministry of Health Malaysia (NMRR ID23-00579-NAR). Permission has also been obtained from the hospital authorities for accessing medical records.

## ACKNOWLEDGMENT

We would like to thank the Director General of Health Malaysia for his permission to publish this article.

## CONCLUSION

This is the first echocardiographic base multicenter study estimating the echocardiographic detection rate of VHD in multiple tertiary care settings in Perak. The echocardiographic detection rate of VHD was 7.5%, of which functional MR is the commonest valve lesion. Majority of patients refused valve intervention due to old age. Our results highlight the evolving landscape of valvular heart disease in Malaysia, marked by a predominance of degenerative and functional etiologies. Echocardiography remains a vital tool in early diagnosis, risk stratification, and treatment planning for patients with VHD.

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# Clinical review of laryngomalacia in a tertiary hospital

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## ABSTRACT

**Introduction:** Laryngomalacia is the most common cause of stridor in infants, with severity ranging from mild to severe forms. Accurate classifications of severity is essential for guiding management and improving outcomes.

**Material and methods:** We conducted a retrospective study of paediatric patients under two years of age diagnosed with laryngomalacia at a tertiary referral centre between January 2010 and December 2020. Data collected included demographic details, clinical presentation, comorbidities, endoscopic findings, treatment, and follow-up duration. Severity was classified using a symptoms-based scoring system by Shah et al, while laryngomalacia types were determined according to Olney et al's endoscopic classification. Association between severity, endoscopic findings, comorbidities and treatment choice were analysed using logistic regression.

**Results:** A total of 148 patients were included (59.49% male). Mild, moderate, and severe laryngomalacia were observed in 45.27%, 35.14%, and 19.59% of patients, respectively. Type 3 laryngomalacia, identified via endoscopy, was significantly associated with severe disease ( $p < 0.001$ ). Comorbidities, particularly gastroesophageal reflux disease, cardiac, pulmonary, syndromic, neurological conditions and synchronous airway lesions, were significantly linked to higher severity ( $p < 0.05$ ). A strong association was found between severity and treatment: moderate cases had 89.6 times, and severe cases 133.3 times, the odds of receiving surgical intervention compared to mild cases ( $p < 0.001$ ).

**Conclusion:** Mild laryngomalacia was most prevalent, but severity increased with specific comorbidities and endoscopic findings. Objective symptom scoring and endoscopic classification are valuable for assessing severity and guiding appropriate management in laryngomalacia.

## KEYWORDS:

*Laryngomalacia, congenital stridor, comorbidities, conservative, supraglottoplasty*

## INTRODUCTION

Laryngomalacia is the leading cause of stridor in infants, accounting for 35-75% of cases.<sup>1</sup> It results from dynamic upper airway obstruction due to inward collapse of supraglottic structures during inspiration. Proposed aetiologies include immature laryngeal cartilage, anatomical variations, gastroesophageal reflux, poor neuromuscular

control, and hypotonia, though none are definitively proven.<sup>2</sup>

Clinically, laryngomalacia presents with high-pitched, musical inspiratory stridor, typically appearing in the first weeks of life, peaking at 6 to 9 months and resolving spontaneously by 12 to 24 months.<sup>3</sup> Severe cases may present with respiratory distress, apnoea, cyanosis, and feeding difficulties, including poor coordination of sucking and swallowing, choking, regurgitation, and micro-aspiration. Severity of laryngomalacia is categorised as mild, moderate, or severe, with severe cases marked by stridor, dyspnoea, feeding problems, cyanosis, and failure to thrive.<sup>1</sup>

Landry and Thompson in 2012 classify laryngomalacia severity based on clinical presentation and oxygen saturation levels: mild laryngomalacia is defined by the presence of inspiratory stridor alone with resting oxygen saturation (SpO<sub>2</sub>) greater than 98%; moderate laryngomalacia is characterised by inspiratory stridor accompanied by frequent feeding difficulties and choking, with SpO<sub>2</sub> between 95-96%; and severe laryngomalacia includes apnoea, cyanosis, failure to thrive and a resting SpO<sub>2</sub> of 85-86%.<sup>3</sup>

In contrast, Shah et al<sup>4</sup> propose an objective symptom-based scoring system, where milder symptoms are assigned a score of 1, and more severe manifestations such as apnoea, cyanosis, or failure to thrive, are given higher scores. The cumulative score categorised severity as mild (1-3), moderate (4-5), or severe (6 or more). The presence of comorbidities also significantly impacts the severity and clinical progression of laryngomalacia. The most frequently encountered comorbidities are gastroesophageal reflux disease (GERD) and neurological disorders.<sup>2</sup> Additional factors such as synchronous airway lesions, congenital heart disease, and the presence of syndrome or genetic disorder may also influence disease outcomes.<sup>2</sup>

Flexible nasopharyngolaryngoscopy (FNPLS) is the gold standard for diagnosis, allowing direct visualization of supraglottic collapse.<sup>2</sup> Olney et al, classified laryngomalacia into three types based on FNPLS: Type 1 (arytenoid mucosa prolapse), Type 2 (foreshortened aryepiglottic folds) and Type 3 (posterior epiglottic displacement).<sup>5</sup> Most infants (80%) have mild to moderate symptoms managed conservatively and resolved spontaneously at the age of 12 to 24 months, while up to 20% with severe symptoms may require surgical intervention.<sup>1</sup>

This study aimed to determine the severity distribution of laryngomalacia using symptoms score and to explore the

*This article was accepted: 11 June 2025*

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associations between severity, endoscopic findings, comorbidities, and treatment in a tertiary centre cohort. Despite the existence of several classification systems, there is limited research that systematically correlates objective symptoms severity scoring with anatomical findings, comorbidities and management outcomes in laryngomalacia. This gap can lead to inconsistent severity assessment and suboptimal treatment selection, potentially affecting patient outcomes. By applying an objective scoring system and correlating it with clinical and endoscopic data, this study seeks to enhance the precision of severity assessment and guide evidence-based management.

## MATERIALS AND METHODS

This retrospective study was conducted at Hospital Pakar Universiti Sains Malaysia (HPUSM), Kelantan, following approval from the Human Research Ethics Committee (USM/JEPeM/21030205). We included paediatric patients under two years of age who were diagnosed with laryngomalacia between January 2010 and December 2020. Inclusion criteria were diagnosis of laryngomalacia established by 24 months of age and availability of complete clinical and endoscopic records. Patients with a history of prior airway surgery were excluded. Data collected included demographic and perinatal details (gender, gestational age, APGAR score, birth weight, age at presentation and diagnosis, antenatal history), presenting symptoms, disease severity, comorbidities (including syndromic and non-syndromic conditions) endoscopic findings, presence of synchronous airway lesions, treatment modalities, and duration of follow-up. All surgical operations were performed under the Paediatric Otorhinolaryngology service, by one of three designated Pediatric ORL surgeons in HPUSM.

Severity was classified using Shah et al.'s scoring system: mild (score 1-3), moderate (4-5), severe (6 or more)<sup>4</sup>. Laryngomalacia types were assigned per Olney et al. based on FNLPSS. Statistical analysis was performed using IBM Statistical Package for Social Sciences (SPSS version 27.0) and STATA 16. Ordinal logistic regression assessed associations between clinical features and severity. Simple logistic regression analysed the link between severity and treatment. Significant was set at p-value less than 0.05.

## RESULTS

A total of 148 patients met inclusion criteria (59.46% male). Table I shows the details of the descriptive analysis of variables involved in the study. The most common symptoms was stridor. Followed by retraction/ chest recession, feeding difficulties, dyspnoea, failure to thrive, cyanosis and apnoea.

From Table II below, the severity distribution were mild (45.27%), moderate (35.14%) and severe (19.54%).

Detailed endoscopy revealed the following types of laryngomalacia: Type 1 in 12 patients (8.1%), Type 2 in 72 patients (48.6%), and combined types in 64 patients (43.2%). The combined types were accounted for Type 1+2 in 50 patients, Type 2+3 in 8 patients, Type 1+3 in 1 patient and Type 1+2+3 in 5 patients. Isolated Type 3 was not observed;

however, Type 3 in combination was significantly associated with severe form of laryngomalacia ( $p < 0.001$ ).

Table III shows an association between the type and severity of laryngomalacia. Comorbidity was found in 84 patients (56.76%). The most commonly found comorbidity was GERD followed by syndromic, cardiac comorbidity, neurological disease, SAL, lung comorbidity, renal disease, and GIT disease as shown in Table I. Other comorbidities such as prematurity, hypothyroidism, asymmetrical small for gestational age with microcephaly, cleft lip and palate were found in 20 patients (13.52%). Patients who had cardiac, lung, neurological disease, GERD, syndromic, and SAL were found to have a significant association with the severity of laryngomalacia. However, renal disease and GIT disease showed no association with the severity of laryngomalacia.

Table IV shows the association between comorbidities and the severity of laryngomalacia. Comorbidities were present in 56.76%, most commonly GERD, followed by syndromic, cardiac, neurological, synchronous airway lesions, pulmonary, renal and gastrointestinal diseases. Cardiac, pulmonary, neurological, GERD, syndromic conditions and synchronous airway lesions were significantly associated with increased severity ( $p < 0.05$ ).

Table V demonstrates the association between the severity of laryngomalacia and the treatment of choice among patients. Treatment of choice was significantly associated with severity. Moderate cases had 89.60 times, and severe cases 133.33 times, the odds of surgical intervention compared to mild cases ( $p < 0.001$ ). Surgical interventions included aryepiglottoplasty ( $n=53$ ), aryepiglottoplasty with tracheostomy ( $n=12$ ), and aryepiglottoplasty with epiglottopexy ( $n=4$ ).

## DISCUSSION

Laryngomalacia was more prevalent in males (male:female ratio of about 1.5:1), consistent with other studies.<sup>6,7,8</sup> Inspiratory stridor was universal<sup>4,5,7</sup> and nearly half of patients (45.89%) had feeding problems, a known risk for poor weight gain due to increased respiratory effort, gastroesophageal or laryngopharyngeal reflux disorders, and uncoordinated suck-swallow-breathing.

From our study, Type 3 in endoscopic findings correlated with severe disease and surgical intervention, aligning with prior reports.<sup>9-10</sup> All Type 3 cases were combined with other types and managed surgically, often with supraglottoplasty and epiglottopexy. This involves surgical division of the short aryepiglottic folds to release the epiglottis and the removal of excess aryepiglottic fold mucosa to prevent it from prolapsing over the glottis as well as fixation of the epiglottis to the base of the tongue.

Over half of patients had comorbidities, most commonly GERD, which was significantly associated with severe symptoms, as reported in other studies.<sup>1,3,11,12,13</sup> Reflux disease had been reported in 65%-100% of infants with laryngomalacia.<sup>5,14</sup> Infants with laryngomalacia experience increased airway resistance, which leads to higher negative

Table I: Descriptive analysis of demographics, symptoms, and co-morbidities

Variable	n (%)
<b>Demographic</b>	
Gender	
Male	88 (59.46)
Female	60 (40.54)
Gestational age (Weeks)	38.00 (2.00) <sup>a</sup>
Age of presentation (Days)	14.00 (23.00) <sup>a</sup>
Age of diagnosis (Days)	30.00 (46.00) <sup>a</sup>
Birth weight (g)	2900.00 (800.00) <sup>b</sup>
APGAR score at 1 minute	9.00 (0.50) <sup>a</sup>
APGAR score at 5 minutes	10.00 (0.00) <sup>a</sup>
Variable	n (%)
<b>Symptoms</b>	
Stridor	148 (100.00)
Retraction/Chest recession	134 (90.54)
Feeding difficulties	67 (45.27)
Failure to thrive	35 (23.65)
Dyspnea	62 (41.89)
Apnea	7 (4.73)
Cyanosis	15 (10.14)
<b>Co-morbidities</b>	
Cardiac disease	30 (20.27)
Lung disease	11 (7.43)
Neurological disease	30 (20.27)
Syndromic	32 (21.62)
GERD	46 (31.08)
Gastro intestinal disease (GIT)	2 (1.35)
Renal disease	4 (2.70)
Synchronous airway lesion (SAL)	25 (16.89)
Others	20 (13.51)
None	64 (43.24)

<sup>a</sup> Median (IQR)<sup>b</sup> Mean (SD)

Table II: Proportion of severity of laryngomalacia among study population

Severity	n (%)
Mild	67 (45.27%)
Moderate	52 (35.14%)
Severe	29 (19.54%)

Table III: Association between type and severity of laryngomalacia

Variable	Regression coefficient (b)	Wald z test	Crude OR (95% CI)	p-value
Type 1	-0.06	-0.13	0.95 (0.42,2.14)	0.893
Type 2	0.36	0.65	1.44 (0.48,4.30)	0.515
Type 3	2.84	4.53	17.08 (5.00,58.38)	<0.001

intrathoracic pressures during inspiration. This pressure gradient can compromise the protective function of the oesophageal sphincters, increasing the risk of gastroesophageal reflux. The resulting reflux may irritate the laryngeal mucosa, causing oedema and further exacerbating airway collapse.<sup>15</sup>

Additionally, symptoms such as coughing, choking, and aspiration observed in infants with moderate to severe laryngomalacia are often attributed to GERD, which impairs laryngeal sensation and disrupts airway protection and

swallowing mechanism.<sup>16-18</sup> Therefore, it is recommended that all laryngomalacia patients with feeding difficulties be treated for GERD. Acid suppression therapy has been shown to improve laryngeal sensation, enhance swallowing function, and potentially shorten the disease course.<sup>5</sup> However, if severe symptoms persist despite optimal medical management, surgical intervention should be considered.<sup>15</sup> Persistent partial airway obstruction can generate significant pressure differences between the thoracic and abdominal cavities, further weakening the lower oesophageal sphincter and perpetuating GERD. In our cohort, 36 out of 46 patients

Table IV: Association between comorbidities and severity of laryngomalacia

Variable	Regression coefficient (b)	Wald z test	Crude OR (95% CI)	p-value
Cardiac disease	0.88	2.28	2.41 (1.13, 5.13)	0.023
Lung disease	2.03	3.08	7.63 (2.09, 27.82)	0.002
Neurological disease	1.09	2.75	2.98 (1.37, 6.47)	0.006
Syndromic	1.61	4.07	4.99 (2.30, 10.80)	<0.001
GERD	2.41	6.09	11.14 (5.13, 24.21)	<0.001
Synchronous airway lesion	2.19	5.03	8.96 (3.81, 21.04)	<0.001
Renal disease	1.29	1.30	3.64 (0.52, 25.41)	0.192
GIT disease	0.62	0.40	1.85 (0.09, 37.76)	0.688

Table V: Association between severity of laryngomalacia and treatment of choice among patients

Variable	Treatment n (%)		b	Wald test	OR (95% CI)	p
	Conservative	Surgical				
Severity						
Mild	64 (82.1)	3 (4.3)	0		1	
Moderate	10 (12.8)	42 (60.0)	4.49	6.54	89.60 (23.28, 344.81)	<0.001
Severe	4 (5.1)	25 (35.7)	4.89	6.12	133.33 (27.83, 638.77)	<0.001

with GERD symptoms had moderate to severe laryngomalacia and all underwent supraglottoplasty. This consistent with finding by Hadfield et al., who reported that supraglottoplasty can reduce gastroesophageal reflux in patients with laryngomalacia.<sup>15</sup>

The prevalence of syndromic and genetic disorders among laryngomalacia patients has been reported to range from 8% to 20%.<sup>5,15</sup> The most frequently observed syndromes in the literature include Down Syndrome, CHARGE syndrome, and Pierre Robin sequences.<sup>19</sup> Similarly, in our study, Down syndrome was the most common, present in 10 patients, followed by unclassified syndromes in 7 patients and Pierre Robin sequence in 4 patients. Notably, syndromes associated with retrognathia, such as Pierre Robin sequence, were more likely to require tracheostomy; in our study, 2 out of 4 patients with Pierre Robin sequence underwent tracheostomy. These findings indicate that syndromic and genetic abnormalities are significantly associated with increased severity of laryngomalacia.

Synchronous airway lesion (SAL) are reported in 12 to 45% of laryngomalacia cases, with tracheomalacia being the most common, followed by subglottic stenosis and vocal cord paralysis.<sup>20</sup> Studies has shown that the presence of SAL is associated with greater disease severity and an increased likelihood of requiring surgery.<sup>21,22</sup> In our series, all patients with SAL had severe laryngomalacia and required surgical intervention. However, some reports suggest that SAL may not always correlate with clinical severity.<sup>23</sup>

The management of laryngomalacia is guided by disease severity. Mild cases typically require only observation, while moderate cases with feeding issues may benefit from anti-reflux therapy and, in some instances, surgical intervention. Surgery is reserved for severe cases presenting with failure to thrive or respiratory distress.<sup>9</sup> Our findings align with previous studies, demonstrating a significant association between laryngomalacia severity and treatment modality.<sup>5,9,24</sup> Nearly all patients with mild laryngomalacia were managed conservatively, whereas 67 patients with moderate or severe

disease underwent supraglottoplasty, with or without epiglottopexy.

This study supports the utility of an objective scoring system for classifying disease severity and guiding appropriate treatment selection, ultimately improving patient outcomes and addressing a critical gap in standardized assessment. Our findings align with global prevalence rates (mild: 45.3%, moderate: 35.1%, severe: 19.6%) but uniquely highlight the predictive value of endoscopic typing (Type 3) and comorbidities (GERD, SAL, Syndromic conditions) in severe progression. The strong association between severity and surgical likelihood (moderate: OR 89.6; severe OR 133.3) underscores the scoring system's clinical relevance, supporting its adoption for risk stratification.<sup>4</sup>

## CONCLUSION

In summary, mild laryngomalacia was the most prevalent form observed in our study, followed by moderate and severe cases. Disease severity was significantly influenced by the presence of comorbidities, particularly GERD, syndromic and genetic disorders and synchronous airway lesions. The anatomical type of laryngomalacia, as determined by endoscopic evaluation, was also associated with clinical severity. Our findings demonstrate that an objective symptom-based scoring system is effective for classifying laryngomalacia severity and guiding management, ensuring that treatment is appropriately matched to disease severity.

## ACKNOWLEDGEMENT

The authors would like to thank all staff of the Medical Record of Universiti Sains Malaysia for their contribution to this study. There is no conflict of interest regarding the publication of this study.

## DISCLOSURE OF FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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# Comparing the effectiveness of retina funduscopy using direct ophthalmoscope and handheld non-mydratic digital retina fundus camera in medical practice

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## ABSTRACT

**Introduction:** Retinal examination plays an essential role in ocular assessment and serves as a key tool for diagnosing various eye conditions. The traditional direct ophthalmoscope (DO) remains widely used due to its affordability, accessibility, and rapid application. However, it presents several limitations, including a steep learning curve, narrow field of view, and strong dependence on user expertise. In contrast, the handheld fundus camera (HFC) is a newer innovation that provides high-resolution digital imaging and is more user-friendly. This study compared the usability, diagnostic confidence, and user preference between the DO and HFC among medical students and practitioners.

**Materials and Methods:** This quasi-experimental study was conducted among 70 participants comprising medical students and practitioners. All participants had prior exposure to the DO and were recruited through convenience sampling. The study took place at the Faculty of Medicine and Health Sciences, Universiti Sains Islam Malaysia (USIM), Negeri Sembilan and USIM's mobile eye screening sites. Participants received a 15-minute hands-on training with both the DO (Welch Allyn) and HFC (New Eyes) before performing non-mydratic retinal examinations on separate patients. A structured questionnaire was used to assess device usability, comfort, diagnostic confidence, ability to identify retinal structures, and System Usability Scale (SUS) scores. Data were analysed using paired t-tests, Fisher's exact test, and chi-square tests.

**Results:** Among the participants, 70% were female and 62.9% were medical students. The mean(SD) time to identify retinal structures was significantly shorter with the HFC (2.8(1.84) minutes) compared to the DO (6.4(5.77) minutes;  $p < 0.001$ ). While red reflex detection was higher with the DO (97.1%), the HFC significantly outperformed in locating key structures: optic nerve (88.6% vs. 42.9%,  $p < 0.001$ ) and fovea (82.9% vs. 41.4%,  $p < 0.001$ ). No significant difference was found in identifying retinal vessels. Confidence in findings (92.9% vs. 32.9%) and image clarity (94.3% vs. 45.7%) favoured the HFC, with both differences being statistically significant ( $p < 0.001$ ). The HFC scored higher in usability with a mean SUS score of 64.0(9.37) versus 58.6(9.91) for the DO ( $p < 0.001$ ). Notably, 97.1% of participants preferred the HFC over the DO.

**Conclusion:** Our findings show that the HFC offers clear advantages over the traditional DO, especially in ease of use, diagnostic confidence, and user experience. These strengths make it a more effective tool in clinical practice and medical education

## KEYWORDS:

*Handheld fundus camera, direct ophthalmoscope, retinal examination, diagnostic confidence, medical education, usability, system usability scale*

## INTRODUCTION

Diagnostic technologies in the ophthalmology field have undergone significant advancements over the past few decades. The innovation has led to the development of new diagnostic tools, which are more accurate, effective, and user-friendly. Consequently, it improves clinical confidence, preference and patient outcomes. The DO and the HFC have emerged as key tools in clinical practice. Invented in 1851 by Hermann von Helmholtz, the DO has long been used for ophthalmic diagnosis among medical practitioners. It is an essential component in the ophthalmology medical curriculum for undergraduate medical students, particularly for examining the fundus.<sup>1,2</sup> Despite its widespread use, effective utilisation of the DO requires significant skill and presents a notable learning curve.<sup>3</sup> This challenge is particularly pronounced among those with limited hands-on experience.

Several studies have reported that many medical practitioners exhibit low confidence when using DO, which may impact diagnoses accuracy and consequently, patient treatment.<sup>4,5</sup> Introduced in the early 20th century, the HFC represents a modern approach to retinal imaging. It captures high-resolution digital images of the retina, enabling more accurate and precise examination.<sup>6</sup> The device is user-friendly and produces superior image quality, which enhances practitioner confidence and contributes to a growing preference for its use.<sup>7</sup> Medical practitioners and students often find the HFC more accessible and cost-effective, especially in educational settings, where ease of use and immediate feedback are crucial for skill development.<sup>8</sup> Retinal fundus viewing is essential for diagnoses, management and prevention of blindness as well as in detecting life-threatening conditions. A thorough understanding of retinal

This article was accepted: 23 June 2025

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anatomy and accurate interpretation of fundus images are fundamental for building diagnostic confidence and providing appropriate management.

Despite the advancements in ophthalmic diagnostic tools, a major challenge remains in ensuring medical professionals and students are adequately trained and confident in using these technologies. Evidence also suggests that user confidence in using diagnostic tools directly correlates with the accuracy of clinical assessments.<sup>4,9</sup> However, the integration of such advanced tools into medical training programs remains inconsistent, and there is a need for comprehensive studies to evaluate their impact on diagnostic performance and clinical outcomes. This study aims to evaluate and compare the image quality, confidence levels and preferences of medical professionals and students in using the DO and the HFC. By highlighting the differences in how each tool is experienced, especially in terms of ease and effectiveness, we hope to offer practical insights that can help improve both teaching and clinical practice.

## MATERIALS AND METHODS

### Ethical Approval

Ethical approval was obtained from the USIM Ethics Committee (grant code: PPPI/FPSK/0122/USIM/14122) and adhered to the Declaration of Helsinki and ICH guidelines for good clinical practice. Written informed consent was obtained from all study participants.

### Participants

Participants, including medical doctors and students, were recruited through convenience sampling. The participants were medical doctors from both private and government facilities, medical officers participating in Universiti Sains Islam Malaysia (USIM) mobile eye screening program in Negeri Sembilan, medical lecturers from the Faculty of Medicine and Health Sciences USIM, and general practitioners. Additionally, medical students from USIM who have completed their ophthalmology postings were also included. Participants must also have prior training with a DO. Ophthalmologists and ophthalmology trainees were excluded from the study.

The study took place at the USIM mobile eye screening sites in Negeri Sembilan and the Faculty of Medicine and Health Sciences, USIM, in Nilai, Negeri Sembilan. The sample size was calculated based on a study conducted in the United States comparing direct ophthalmoscope and digital smartphone ophthalmoscope.<sup>10</sup> A minimum sample size of 70 participants was calculated using Open Epi (95% Confidence Interval, 80% power and significant level of 0.05) based on the success rates of identifying retinal structures using both devices (50.0% for direct ophthalmoscope and 82.3% for digital smartphone ophthalmoscope).<sup>11</sup>

### Study flow

The participants were randomly assigned to use either the DO or the HFC first. They received a 15-minute demonstration and practice session on the assigned device before the evaluation. Each participant conducted a timed retinal fundus examination on a non-dilated normal patient and subsequently complete a questionnaire about their

experience. Afterward, participants were then given other device and assigned to different patients to avoid bias.

The study utilized a DO and a HFC testing device, along with a questionnaire. This questionnaire included basic demographic information as well as the ability to identify ocular structures, confidence of findings, image sharpness, comfort level and device preference for both DO and HFC. The System Usability Scale (SUS) was used to measure comfort level. The SUS is a validated tool for evaluating a product, system or service.<sup>12</sup> Each item is scored using a 10-point Likert scale from "strongly disagree" to "strongly agree". The scoring is calculated by adding all the 10 items scores resulting in a score ranging from 10-100.<sup>12,13</sup>

### Statistical Analysis

Data was analysed using Statistical Package for Social Science (SPSS), version 22.0 (IBM Corp, USA). Continuous data was presented as mean (SD), and categorical data was presented as frequency and percentages (%). A paired t-test was used to compare the duration taken for examination in minutes and the total SUS score between both devices. The ability to visualize retinal structure and confidence level were evaluated using Fisher's exact and chi-square test.

## RESULTS

A total of 70 participants were recruited for the study with the highest being Malays (65%), followed by Indian (4%) and Dusun (1.4%). The majority (70%) of the participants were female and 60% were myopic. The participants were medical practitioners (37.1%) and medical students (62.9%). The medical practitioners consisted of medical lecturers, general practitioners and non-ophthalmology medical officers. The mean(SD) age of the participants was 27.5(6.06) years old. More than half (57.1%) had 5-10 years' experience as medical students and in-service doctors. The majority of the participants (71.4%) used a DO in the past 6 months.

Table II presents a comparison of retinal structure visualization, examination duration, and user confidence between the DO and the HFC. The duration of finding retinal structures was significantly longer using the DO (6.4 minutes(5.77) compared to the HFC (2.8 minutes (1.84)). The DO gave the user higher ability (97.1%) in eliciting red reflex compared to the HFC (64.3%). The ability to find the optic nerve was higher in the HFC at 88.6% compared to 42.9% using the DO. The result was relatively similar to finding the fovea using the DO and the HFC with percentages of 41.4% and 82.9% respectively. However, there was no significant difference in finding the retinal vessel using both equipment. The results showed that users of HFC reported higher confidence of the correct findings (92.9%) and confidence of the sharpness of the image (94.3%).

Table III summarises the results of the SUS scores for both DO and HFC. The HFC outperforms the DO in preference for ease of usage, integrated function, ease of learning and usage confidence. The difference in the SUS scores was statistically significant with a mean difference of 5.39 (Table IV). The HFC was perceived as more user-friendly and gives more confidence to the users.

Table I: Sociodemographic profiles of the participants

Characteristics	n	%
Race		
Malay	65	92.9
India	4	5.7
Dusun	1	1.4
Gender		
Female	49	70
Male	21	30
Refractive Status		
Emmetrope	28	40
Myopia	42	60
Occupation		
Medical Practitioner	26	37.1
Medical Student	44	62.9
Duration of study and employment		
Less until less than 5 years	23	32.9
5-10 years	40	57.1
More than 10 years	6	8.6
Missing	1	1.4
When was the last time you used direct ophthalmoscope?		
Less than 6 months	50	71.4
6 months to less than 1 year	4	5.7
1-2 years	2	2.9
More than 2 years to 3 years	3	4.3
More than 3 years	11	15.7

Table II: Comparison of Retinal Structure Visualization, Examination Duration, and User Confidence Between Direct Ophthalmoscope (DO) and Handheld Fundus Camera (HFC) Among Medical Practitioners

	DO	HFC	p-value
	Mean (SD) N (%)	Mean (SD) p-value	
Duration taken for examination (in minutes)	6.4(5.77)	2.8(1.84)	<0.001 <sup>a</sup>
N (%)			
Able to get red reflex			
Yes	68 (97.1)	45 (64.3)	<0.001 <sup>b</sup>
No	2 (2.9)	25 (35.7)	
Optic nerve			
Yes	30 (42.9)	62 (88.6)	<0.001 <sup>c</sup>
No	40 (57.1)	8 (11.4)	
Retinal vessel			
Yes	64 (91.4)	67 (95.7)	0.49
No	6 (8.6)	3 (4.3)	
Fovea using			
Yes	29 (41.4)	58 (82.9)	<0.001 <sup>c</sup>
No	41 (58.6)	12 (17.1)	
Confident of correct finding			
Yes	23 (32.9)	65 (92.9)	<0.001 <sup>c</sup>
No	38 (54.3)	5 (7.1)	
Confident sharpness of image			
Yes	32 (45.7)	66 (94.3)	<0.001 <sup>c</sup>
No	38 (54.3)	4 (5.7)	

<sup>a</sup>paired t-test, <sup>b</sup>fisher exact test, <sup>c</sup>chi square

In terms of overall preference, 68 (97%) indicated a preference for the HFC, while only 2 (3%) preferred the DO. This strong preference for the HFC underscores its perceived advantages in usability, image clarity, and ease of operation

## DISCUSSION

Retinal examination remains an important part of diagnosing and managing various eye diseases. Although the DO has been used for many years, it still poses

challenges, especially in teaching and routine clinical use. Recent technologies with more user-friendly devices such as HFC have been introduced as they offer better ways to improve the user experience and support learning, particularly for those still in training. Therefore, evidence to support the learning outcomes in the local context is important to justify introducing new devices in teaching.

Gender distribution among the participants showed a predominance of females (70%) compared to males (30%).

**Table III: Comparison of Systems Usability Scale (SUS) Scores Between Direct Ophthalmoscope and Handheld Fundus Camera**

	DO	HFC	Mean difference (SD)	p-value
	Score (1-10) Mean (SD)	Score (1-10) Mean (SD)		
I think I would like to use this system frequently	6.0 (2.78)	9.1 (1.32)	-3.1 (3.00)	<0.001
I found the system unnecessarily complex	5.6 (3.05)	3.6 (2.52)	2.0 (3.26)	<0.001
I thought the system was easy to use	6.1 (2.82)	9.1 (1.36)	-3.0 (3.07)	<0.001
I think that I would need the support of a technical person to be able to use this system	5.4 (3.03)	4.5 (2.70)	0.9 (3.18)	0.016
I found the various functions in this system were well integrated	6.9 (2.28)	8.6 (1.46)	-1.8 (2.34)	<0.001
I thought there was too much inconsistency in this system	5.6 (2.87)	3.4 (3.22)	2.2 (3.22)	<0.001
I would imagine that most people would learn to use this system very quickly	5.4 (2.90)	8.9 (1.54)	-3.5 (3.16)	<0.001
I found the system very cumbersome to use	5.5 (2.86)	3.6 (2.53)	1.81 (3.49)	<0.001
I felt very confident using the system	5.5 (2.82)	8.8 (1.56)	-3.3 (3.06)	<0.001
I needed to learn a lot of things before I could get going with this system	6.7 (2.69)	4.3 (2.69)	2.4 (3.22)	<0.001

paired t-test

**Table IV: Comparison of Total System Usability Scale (SUS) Scores Between Direct Ophthalmoscope and Handheld Fundus Camera**

Total SUS score	Mean score	SD	Mean difference	95% CI	p-value
Direct Ophthalmoscopy	58.6	9.91	-5.39	-7.36, -3.40	<0.001
Handheld fundus camera	64.0	9.37			

paired t-test

This reflects current demographic trends within healthcare training programs. This gender imbalance might influence the comfort and usability ratings of the devices, as different ergonomic preferences could exist between genders. Future studies with more balanced gender representation would help to clarify the potential influence from this gender factor. Secondly, regarding refractive status, 60% of the participants were myopic, while 40% were emmetropic. Myopic participants depend on corrective lenses, which can influence comfort and effectiveness while using HFC. However, no significant difference in device preference or usability was observed between myopic and emmetropic participants in this study.

Apart from that, the recent use of the DO played a role in mastering a new device such as HFC. This can be seen as more than half of the participants (71.4%) reported that they have used DO within the last 6 months. This indicates that most participants are actively engaged in clinical practices where ophthalmoscopy examination is routinely performed and are already familiar with the DO. The user-friendly nature of HFC could be a beneficial addition, especially for those who did not use ophthalmoscopes frequently. This could further enhance the efficiency and comfort in retinal examinations.

In comparing the device performance, the DO showed a higher success rate of red reflex detection (97.1%), however this did not translate into better visualization of deeper retinal structures. The optical properties of DO, including its coaxial illumination aligned with the viewing axis, allows for the pupils to be illuminated as light reflects off the back of the retina and through the aperture of the scope.<sup>14</sup> For the examination of the optic nerve and fovea, the HFC showed

significantly higher rates of optic nerve (88.6% vs. 42.9%) and fovea (82.9% vs. 41.4%) identification with no significant difference in detecting retinal vessels. In contrast, both devices performed similarly for retinal vessel visibility, a finding supported by previous research that highlights the adequacy of both tools for vascular assessments.<sup>10,14</sup> These findings are consistent with previous studies reporting that digital fundus cameras and ophthalmoscopes offer better visibility of key retinal landmarks compared to the DO.<sup>10,16,17</sup>

Beyond image clarity, the HFC also allowed significantly faster completion of retinal examinations (mean 2.8 minutes) than the DO (mean 6.4 minutes). This finding is consistent with previous studies which demonstrated that digital fundus camera and smartphone-based ophthalmoscopes not only allow faster identification of ocular structure, but also generally easier to use.<sup>10,16,18</sup> The HFC allows for examination without pupil dilatation, has autofocus capability, captures and stores images, and has a wide field of view. These features make the retinal examination quicker. While the DO remains valuable for red reflex detection, the HFC provides clearer imaging of key retinal landmarks, supporting more accurate diagnosis and learning. Future integration of red reflex functionality into fundus cameras could further enhance their versatility and clinical application.

Diagnostic confidence and perceived image quality were also significantly higher when using the HFC. A total of 92.9% of participants reported confidence in their findings with the HFC, compared to 32.9% with the DO. Confidence in image sharpness was similarly higher respectively (94.3% vs. 45.7%). This is supported by a study comparing the traditional DO with a non-mydriatic automatic fundus

camera among medical students. None of the students were able to visualize the macula using the DO, whereas all were able to do so with the automatic fundus camera. Moreover, diagnostic accuracy for diabetic retinopathy improved significantly by 100% with the automatic camera versus 40% with the direct ophthalmoscope. Students also expressed greater confidence in identifying basic retinal anatomy using the automatic camera (9.6/10 vs. 6.4/10).<sup>15</sup> The integration of HFCs into ophthalmology teaching could therefore enhance both the learning experience and diagnostic competence of future clinicians.

Building on the usability findings, user preference was also overwhelmingly in favour of the HFC. Most notably, 97.1% of participants preferred the HFC over the DO, with only 2.9% indicating a preference for the latter. This overwhelming preference reflects the cumulative advantage of the HFC in ease of use, image quality, diagnostic confidence, and time efficiency. It also underscores a shift in user expectations toward more modern, digital diagnostic tools. These findings align with previous research showing that non-mydratics fundus cameras produce high-quality images that aid in diagnosis and monitoring with better patient experience.<sup>19,20</sup>

While this study provides valuable insights into the usability and diagnostic confidence associated with the DO and HFC, several limitations must be acknowledged. Firstly, convenience sampling was used to recruit participants, which may have introduced some selection bias. Those who volunteered could have had more interest or prior exposure to eye examinations, potentially influencing their confidence and preference. The study used a specific model of HFC (New Eyes) and a particular brand of DO (Welch Allyn). The results may differ with other models or brands, limiting the applicability across all available devices. Additionally, our assessments were limited to a short-term, post-training evaluation. Long-term use and retention of skills over time were not assessed. Another important limitation is the cost difference between the devices; the HFC is substantially more expensive than the DO, which could pose challenges for widespread adoption, particularly in resource-limited settings or for large-scale implementation in undergraduate medical training.

Based on the limitations of this study, we suggest a few areas for future research and improvement in clinical practice. First, future studies should consider using random sampling and involving a more diverse group of participants with different levels of experience. This would increase the representation and reliability of the study. Secondly, while a brief standardized training session was sufficient for this study, longer-term training or follow-up assessments could help evaluate the retention of skills over time. To improve external validity, future research should also include comparisons involving different models and brands of handheld fundus cameras and direct ophthalmoscopes.

Finally, this study has valuable implications for both teaching and clinical practice. The HFC's intuitive design and diagnostic advantages make it a strong candidate for integration into undergraduate ophthalmology training. Its

digital capabilities support self-directed learning, documentation, and teleconsultation for all essential skills in today's healthcare environment. For clinical settings, especially in community and mobile outreach programs, the HFC provides a reliable and efficient tool for early detection and monitoring of retinal disease.

## CONCLUSION

This study demonstrates a strong preference for the HFC among medical professionals and students, with significantly better performance in image quality, ease of use, and user confidence compared to the DO. The high SUS scores and clear user preference highlight the HFC's potential as a more effective diagnostic tool. These results support the integration of HFCs into undergraduate ophthalmology training and wider clinical practice, particularly in settings where user-friendliness and efficiency are essential. Further research should explore the long-term impact, cost-effectiveness, and influence of these devices on clinical decision-making and patient outcomes.

## ACKNOWLEDGEMENTS

We would like to thank the Faculty of Medicine and Health Sciences, USIM, for their support in conducting this study. This research was funded by the USIM Research Grant (Grant code: PPPI/FPSK/0122/USIM/14122). We are also grateful to all participants who contributed their time and effort to this project.

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# Vitamin D status and its association with asthma control and severity in children

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## ABSTRACT

**Introduction:** Vitamin D has an immunomodulatory effect on innate and adaptive immunity within the body, which partially explains its links to inflammation-induced epithelial changes seen in asthma. Various evidence suggest a potential link between vitamin D deficiency and asthma control and severity. This study aimed to determine the vitamin D levels in asthmatic children and their association with asthma severity and control.

**Materials and Methods:** A cross-sectional prospective study was conducted on 72 children with persistent asthma aged 5 to 18 years old in a tertiary hospital in Kelantan. Asthma severity was assessed using the Global Initiative for Asthma (GINA) guidelines based on treatment given. Asthma control was evaluated using both parent/patient self-administered Asthma Control Test (ACT) or Children's Asthma Control Test (c-ACT) scores accordingly to their age and asthma control GINA classification. Serum 25-hydroxyvitamin D [25(OH)D] levels were measured, with <52 nmol/L defined as deficient and 52-73 nmol/L as insufficient.

**Results:** Of the 72 children with persistent asthma, 40.3% had vitamin D deficient and 38.9% had vitamin D insufficient with a mean 25(OH)D level of 58.74 nmol/L. Being female, older age group and higher body mass index (BMI) were associated with vitamin D deficiency ( $p = <0.05$ ). Lower vitamin D levels were significantly associated with increased asthma severity ( $p = 0.020$ ). There was no significant association between vitamin D levels and asthma control based on GINA classification ( $p = 0.470$ ) and c-ACT/ACT scores ( $p = 0.052$ ).

**Conclusion:** High prevalence of vitamin D deficient and insufficient was found among children with persistent asthma. Vitamin D deficiency was significantly associated with increased asthma severity. This group of children needs further evaluation for intervention. We recommend routine assessments of vitamin D levels among children with moderate to severe asthma.

## KEYWORDS:

*Asthma control, asthma severity, children, 25-hydroxyvitamin D, vitamin D*

## INTRODUCTION

According to the World Health Organization (WHO), approximately about 339 million people suffered from

asthma globally and the prevalence continues to rise, particularly among children.<sup>1</sup> Despite advancements in asthma management, achieving optimal control and preventing exacerbations remain ongoing challenges.<sup>2</sup> The global burden of asthma has increased significantly over the past 40 years, particularly among children.<sup>3</sup> This rise in asthma prevalence and severity poses a major public health challenge, necessitating the identification of new risk factors and the development of more effective management strategies. In the Asia-Pacific region, the Asthma Insights and Reality in Asia Pacific (AIRIAP) Phase II study reported that 53.4% of patients under 16 years old had uncontrolled asthma.<sup>4</sup> This high prevalence of uncontrolled asthma highlights the urgent need for improved asthma management approaches in this region. Uncontrolled asthma can lead to frequent exacerbations, hospitalizations, and a decreased quality of life for affected children and their families.<sup>5</sup>

Vitamin D plays a pivotal role in the inflammation pathway by regulating the production of inflammatory cytokines and modulating the activity of various immune cells.<sup>6</sup> This regulatory function is particularly relevant in the pathogenesis of inflammatory diseases, including asthma.<sup>6</sup> Research has shown that children with vitamin D deficiency are at a significantly higher risk for asthma, with one study indicating a 6.3-fold increase in risk compared to those with sufficient vitamin D levels.<sup>7</sup> Vitamin D not only serves as a potential predictor for the development of asthma but also influences its severity and control.<sup>8,9,10</sup> Vitamin D is synthesized in the skin upon exposure to ultraviolet B (UVB) radiation from sunlight and can also be obtained from dietary sources such as fish oils, fish, liver, egg yolks and supplements.<sup>10</sup> Vitamin D status was most accurately determined by measuring 25-hydroxyvitamin D since it is the highest of all metabolites and has half-life of approximately three weeks.<sup>10</sup> Vitamin D deficiency is prevalent globally, even in regions with ample sunlight, due to factors such as limited sun exposure, dietary insufficiency, and cultural practices.<sup>10</sup>

The prevalence of vitamin D deficiency and insufficiency among asthmatic children is alarmingly high.<sup>8-10</sup> A study among urban American youth found that 86% of children with persistent asthma had insufficient or deficient vitamin D levels.<sup>9</sup> Similarly, high prevalence rates have been reported in tropical countries, where adequate sunlight exposure would theoretically prevent vitamin D deficiency.<sup>11</sup> This paradox highlights the need for further investigation into underlying

*This article was accepted: 23 June 2025*

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factors contributing to vitamin D deficiency in these populations.

Deficiency in vitamin D has been associated with increased asthma exacerbations, heightened airway inflammation, decreased lung function, and poor asthma control in children.<sup>12</sup> Additionally, studies have shown that children with moderate to severe persistent asthma have significantly lower vitamin D levels compared to those with intermittent asthma.<sup>13</sup> The beneficial effects of vitamin D on asthma may be explained by its role in enhancing glucocorticoid responsiveness, promoting regulatory immune cell phenotypes, reducing infection rates, mitigating airway remodelling, and lowering eosinophilia and IgE levels.<sup>14</sup> However, some studies have reported conflicting results, showing either no effect or adverse effects of vitamin D on asthma incidence and severity.<sup>10-12</sup> This inconsistency makes the relationship between vitamin D levels and asthma unclear.

Surprisingly, vitamin D deficiency is prevalent even in tropical countries with abundant sunlight. In Malaysia, for example, a study among adults in Kuala Lumpur found that 67.4% had vitamin D deficiency, with Indian participants showing the highest prevalence.<sup>15</sup> Similar findings were shown among children and adolescents. A significant study involving 1361 adolescents aged 12 to 13 years old in Perak, Selangor, and Kuala Lumpur found that 78.9% had vitamin D deficiency, with only 7.4% had adequate levels of vitamin D.<sup>16</sup> The South-East Asian Nutrition Survey (SEANUTS) also reported that 47.5% of children aged four to twelve years had low vitamin D levels, with a higher prevalence among girls (54.1%) compared to boys (41.1%).<sup>17</sup>

To date there is no local data available on the association between vitamin D deficient/insufficient with asthma severity and asthma control in Malaysian children. It is important to identify asthmatic children with vitamin D deficient/insufficient as supplementation of vitamin D as an adjunct treatment may help to improve the control of asthma.

## MATERIALS AND METHODS

The study protocol received approval from the Medical Research & Ethics Committee Kementerian Kesihatan Malaysia (NMRR ID-22-02416-RVC (IIR)) and Research Ethics Committee Universiti Kebangsaan Malaysia (JEP-2022-694). All participants or their guardians provided informed consent and assent.

### Study design and participants

This cross-sectional prospective study was conducted at Paediatric clinic Hospital Raja Perempuan Zainab II (HRPZII), Kota Bharu, Kelantan from December 2022 to December 2023. The inclusion criteria included children with persistent asthma, aged 5 to 18 years, on preventer medications for at least 3 months duration. Exclusion criteria comprised children who were taking vitamin D supplements or had other chronic conditions such as other respiratory disease, congenital heart disease with heart failure, malabsorption, malignancy, chronic haematological disease,

immunological disease, chronic kidney disease, or liver disease.

We recorded the demographic data of the study population, including age, gender, Centers for Disease Control (CDC) body mass index (BMI) for age percentile, parents' education level, and household income. Factors affecting vitamin D levels, such as weekly sunlight exposure, duration of physical activity, and weekly dietary intake of vitamin D-enriched foods, were also noted via direct interview using a questionnaire. Vitamin D-enriched foods include eggs, oily fish, cheese and mushrooms.

Asthma severity was determined from the level of treatment required to control symptoms and exacerbations based on the Global Initiative for Asthma (GINA) asthma treatment strategy 2023.<sup>18</sup> Data on current medication required to control symptoms and exacerbations were recorded and the severity of asthma was assessed by the attending clinician. Participants were categorised into mild persistent, moderate persistent and severe persistent asthma.

Asthma control was determined by both GINA classification criteria via direct interview the researcher and parents/patient self-administered Asthma Control Test (ACT) questionnaire<sup>19</sup> for children more than 12 years old, or Childhood Asthma Control Test (c-ACT) questionnaire<sup>20</sup> for children aged 4-11 years old. The questionnaire was based on the symptoms over the last 4 weeks. Additionally, the number of hospitalisations and Emergency Department visits due to asthma in the past 12 months was recorded. Asthma control was classified into well-controlled, partly controlled, and uncontrolled asthma.

Blood samples were obtained to measure serum 25-hydroxyvitamin D [25(OH)D] levels and bone profile parameters, including serum calcium (Ca), phosphate (PO<sub>4</sub>), alkaline phosphatase (ALP), and magnesium (Mg). Since the Pathology Laboratory at HRPZ II couldn't process the 25(OH)D levels, the samples were stored at 2-8°C and transported to the Pathology Laboratory at Hospital Putrajaya for analysis twice a week. The results were then uploaded into the Patient Management System (SPPv3) application. Vitamin D status was classified as deficient (<52 nmol/L), insufficient (52-73 nmol/L), or sufficient (74-250 nmol/L).<sup>21</sup>

Those with sun exposure during 10 a.m. to 3 p.m. of at least 6 hours per week in the previous month were considered as having adequate sun exposure.<sup>22</sup> Physical activity of at least 1 hour daily in the previous month were considered as adequate.<sup>22</sup>

### Statistical analysis

All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) version 29.0. Means and standard deviations or median and interquartile range (IQR) were reported for continuous variables (e.g., age, serum 25(OH)D levels). Frequencies and percentages were reported for categorical variables (e.g., gender, vitamin D status categories). Different characteristics between the groups of the level of asthma control and asthma severity were assessed

**Table I Demographic and clinical data of the study population**

		Subjects (n=72)
Age in years, median (IQR)		8.0 (5)
Gender, n (%)	Male	40 (55.6)
	Female	32 (44.4)
BMI for age percentiles, n (%)	Underweight	14 (19.4)
	Healthy	41 (56.9)
	Overweight	7 (9.7)
	Obese	10 (13.9)
Parents education level, n (%)	Primary	1 (1.4)
	Secondary	33 (45.8)
	Tertiary	38 (52.8)
Household income, n (%)	<RM4000/month	46 (63.9)
	>RM 4000	26 (36.1)
Sun exposure per week, n (%)	<6 hours	65 (90.3)
	>6 hours	7 (9.7)
Physical activity per day, n (%)	<1 hour/day	39 (54.2)
	>1 hour/day	33 (45.8)
Enriched vitamin D containing food, times/week, mean (SD)		2.94 (1.91)
GINA classification of asthma severity, n (%)	Mild persistent	16 (22.2)
	Moderate persistent	44 (61.1)
	Severe persistent	12 (16.7)
Medications used for asthma, n (%)	ICS alone	33 (45.8)
	ICS + LABA	14 (19.4)
	ICS + LTRA	13 (18.1)
	ICS + LABA + LTRA	8 (11.1)
	ICS + LABA + LTRA +Biologics	4 (5.6)
GINA classification of asthma control, n (%)	Well controlled	51 (70.8)
	Partly controlled	18 (25)
	Uncontrolled	3 (4.2)
ACT/c-ACT score asthma control, n (%)	Well controlled	60 (83.3)
	Partly controlled	10 (13.9)
	Uncontrolled	2 (2.8)
Hospitalisations in past 12 months, n (%)	0 time/year	54 (75)
	1 time/year	18 (25)
ED visits past 12 months, n (%)	0 time/year	35 (48.6)
	1 time/year	29 (40.3)
	>2 times/year	8 (11.1)
Vitamin D status, n (%)	Deficient	29 (40.3)
	Insufficient	28 (38.9)
	Sufficient	15 (20.8)

n, numbers; SD, standard deviation; IQR, interquartile range; RM, ringgit Malaysia; BMI, body mass index; ED, emergency department; ICS, inhaled corticosteroids; LABA, long-acting Beta agonist; LTRA, leukotriene receptor agonists.

**Table II: Risk factors of vitamin D deficiency**

Factors	Category	Total	Vitamin D status			p
			Deficient n (%)	Insufficient n (%)	Sufficient n (%)	
Gender	Male	40	10 (25)	17 (42.5)	13 (32.5)	0.002*
	Female	32	19 (59.4)	11 (34.4)	2 (6.3)	
Age in years		72	11 (5)#	8 (3)#	6 (2)#	0.001*
BMI-for-age percentile	Underweight	14	3 (21.4)	9 (64.3)	2 (14.3)	0.010*
	Healthy	41	13 (31.7)	16 (39)	12 (29.3)	
	Overweight	7	6 (85.7)	0 (0)	1 (14.3)	
	Obese	10	7 (70)	3 (30)	0 (0)	
Enriched vitamin D containing food, times/week		72	3(1)#	3(3.5)#	3(2)#	0.626
Parents education level	Primary	1	0 (0)	0 (0)	1 (100)	0.488
	Secondary	33	13 (39.4)	14 (42.4)	6 (18.2)	
	Tertiary	38	16 (42.1)	14 (36.8)	8 (21.1)	
Household income per month	<RM2000	31	11 (35.5)	11 (35.5)	9 (29)	0.682
	RM2000-4000	15	7 (46.7)	6 (40)	2 (13.3)	
	>RM4000	26	11 (42.3)	11 (42.3)	4 (15.4)	
Sun exposure per week	<6 hours	65	26 (40)	24 (36.9)	15 (23.1)	0.160
	>6 hours	7	3 (42.9)	4 (57.1)	0 (0)	
Physical activity per day	<1 hour	39	16 (41)	17 (43.6)	6 (15.4)	0.425
	>1 hour	33	13 (39.4)	11 (33.3)	9 (27.3)	

\*Significant, p<0.05  
#median (IQR)

**Table III: Association vitamin D status and asthma severity/control**

Factors	Category	Total	Vitamin D status			P
			Deficient n (%)	Insufficient n (%)	Sufficient n (%)	
GINA classification of asthma severity	Mild persistent	16	3 (18.8)	5 (31.3)	8 (50)	0.020*
	Moderate persistent	44	19 (43.2)	20 (45.5)	5 (11.4)	
	Severe persistent	12	7 (58.3)	3 (25)	2 (16.7)	
GINA classification of asthma control	Well controlled	51	19 (37.3)	19 (37.3)	13 (25.5)	0.470
	Partly controlled	18	8 (44.4)	8 (44.4)	2 (11.1)	
	Uncontrolled	3	2 (66.7)	1 (33.3)	0 (0)	
ACT/c-ACT score asthma control	Well controlled	60	23 (38.3)	22 (36.7)	15 (25)	0.052
	Partly controlled	10	4 (40)	6 (60)	0 (0)	
	Uncontrolled	2	2 (100)	0 (0)	0 (0)	
Hospitalisations in past 12 months	0 time	54	22 (40.7)	21 (38.9)	11 (20.4)	0.983
	1 time	18	7 (38.9)	7 (38.9)	4 (22.2)	
ED visits past 12 months	0 time	35	14 (40)	13 (37.1)	8 (22.9)	0.955
	1 time	29	11 (37.9)	12 (41.4)	6 (20.7)	
	>2 times	8	4 (50)	3 (37.5)	1 (12.5)	

\*Significant, p<0.05

by the Kruskal-Wallis test (for non-parametric data), and the chi-square test (for descriptive analysis). Pearson’s correlations were used to determine the relationship between two continuous variables. A p-value of <0.05 was considered statistically significant.

Based on previous study by Turkeli et al.<sup>23</sup>, a total of 72 study subjects were required to achieve 80% power of the study and a significance level of 0.05, taking into consideration of 20% dropout rate.

**RESULTS**

A total of 75 Malay children were recruited for the study. However, 3 blood samples with serum 25-hydroxyvitamin D levels were rejected, thus were excluded from the study. This was due to the unavailability of reagents for measuring serum 25- hydroxyvitamin D in the Pathology Laboratory at Hospital Putrajaya at the end of the study recruitment period. Hence, the final total number of participants in this study was 72 children.

**Demographic and Characteristics of Participants**

The median age was 8 years (IQR: 5), with a slight male predominance. Most of the parents (98.6%) had either secondary or tertiary education. About two-thirds of the study population had a total household income of less than RM4000. Many of the children had less than 6 hours of sun exposure per week (90.3%) and less than 1 hour of physical activity per day (54.2%). Half of the children had normal BMI for age percentiles. Over 60% of the children had moderate persistent asthma, with the majority of patients having well-controlled asthma based on both GINA classification and ACT/c-ACT scores, 70.3% and 83.3% respectively. Despite a high proportion of children with well-controlled asthma, 25% of the children experienced asthma-related hospitalizations and 51.4% of them had at least one emergency department visit in the past 1 year. Four patients received biologics as part of their asthma treatment, which includes Dupilumab and Omalizumab. A high percentage of vitamin D deficient (40.3%) and vitamin D insufficient (38.9%) were noted. These are presented in Table I.

**Risk Factors of Vitamin D Deficiency**

Asthmatic female children were found to be vitamin D deficient (59.4%) compared to males (p =0.002). There was a statistically significant difference in age and overall BMI across vitamin D status (p =<0.05). Underweight children had lower rates of vitamin D deficiency compared to overweight and obese children. Children with asthma in the deficient group have a significantly higher median age (11 years, IQR: 5) compared to those in the insufficient (8 years, IQR: 3) and sufficient groups (6 years, IQR: 2). Other factors were not statistically significant. (Table II)

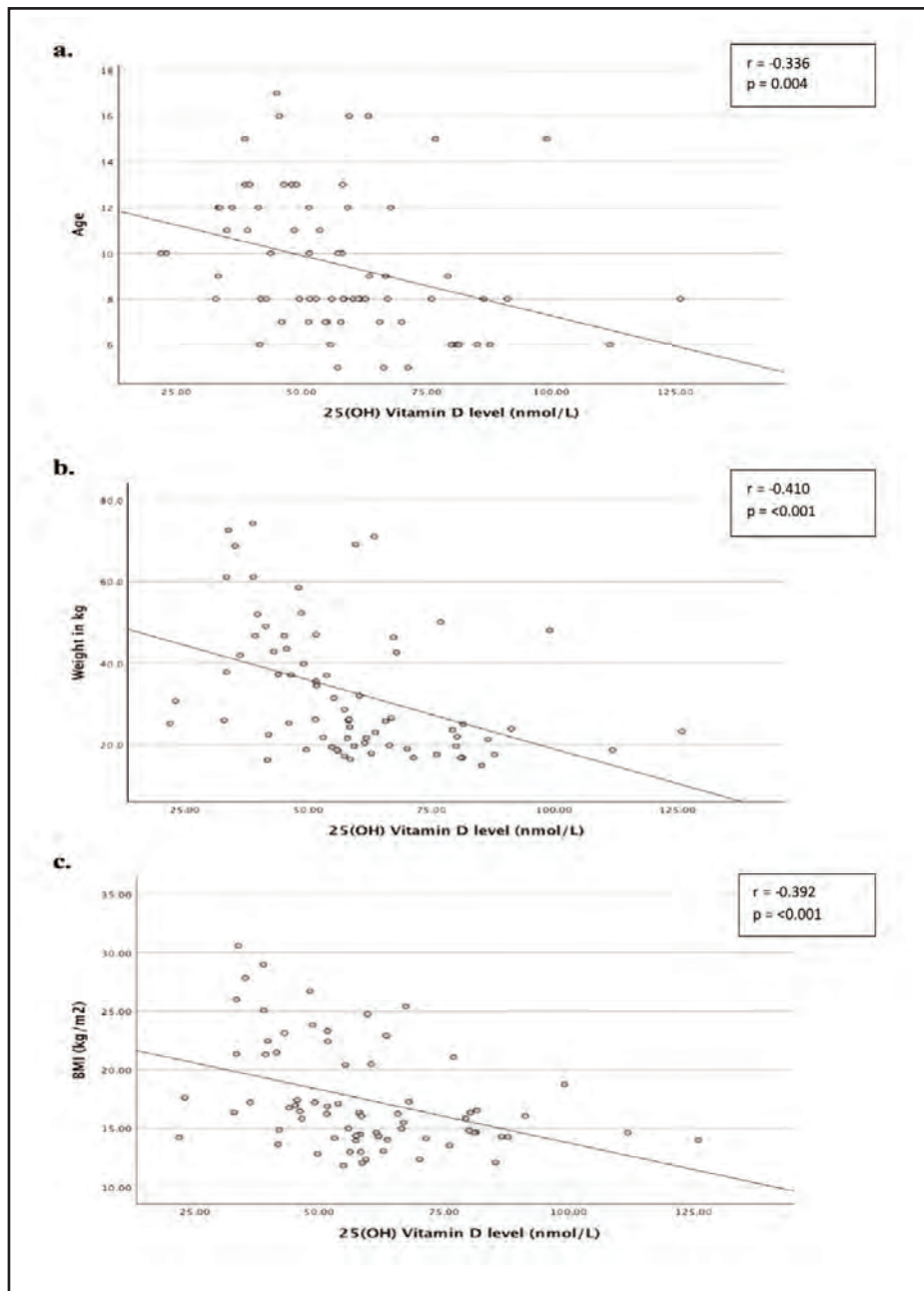
Vitamin D levels were found to be negatively correlated with age (r = -0.336), weight (r = -0.410) and BMI (r = -0.392) among the study participants (Figure 1). The median serum vitamin D levels of mild persistent, moderate persistent and severe persistent groups were 73.72, 55.98 and 48.59ng/mL, respectively (Figure 2). Children with severe persistent asthma have significantly lower median vitamin D levels compared to those with mild asthma (p = <0.05). The plasma levels of calcium, phosphate, magnesium, and alkaline phosphatase were within normal range in all groups of vitamin D status.

**Vitamin D and asthma control/severity**

Vitamin D deficiency was significantly associated with increased severity of asthma (p = <0.05). The majority of the children with moderate persistent asthma were either vitamin D deficient or insufficient (n = 39). However, there was no statistically significant association between vitamin D status and asthma control when evaluated by both ACT/c-ACT score (p=0.052) and GINA classification symptoms (p=0.470). Additionally, no significant association between vitamin D status and the number of hospitalisations and ED visits was found. (Table III)

**DISCUSSION**

Our results showed a high percentage (79.2%) of vitamin D deficiency and insufficiency among Malaysian children with asthma. These findings are consistent with previous research in the neighbouring country, Thailand, which showed 64%



**Fig. 1:** Correlation plots between vitamin d level and age (a), weight (b) and BMI (c)

children with asthma had vitamin D deficient/insufficient.<sup>11</sup> Higher prevalence was also found in Western country among American asthmatic children (86%).<sup>9</sup> Our study found that the mean level of vitamin D among asthmatic children was 58.7 nmol/L. Similar findings were found by Turkeli et al. which showed the mean of serum hydroxyvitamin D among asthmatic children was 55nmol/L compared to children without asthma (80nmol/L).<sup>23</sup> There was no local data on the prevalence of vitamin D among asthmatic children in Malaysia. However, a high prevalence of vitamin D deficiency was found among school aged Malaysian children in the previous study not related to asthma.<sup>16</sup> Taken together, it is implied that vitamin D deficient or insufficient is a serious problem in Malaysia as a tropical country with

abundant sun exposure. The inadequate sun exposure, limited physical activity and lack of vitamin D rich food intake data among the study participants may contribute to the high prevalence of vitamin D deficient/insufficient in this study. Our hot and humid weather also render Malaysian children to avoid outdoor activities during the day.

We found that vitamin D deficiency was associated with female gender, older age and higher BMI in children. Research on vitamin D levels suggests that females are at higher risk of vitamin D deficiency due to various factors.<sup>15,17</sup> These include biological differences such as hormonal influences, lifestyle behaviours like limited sun exposure, and cultural practices, with their clothing habits, especially

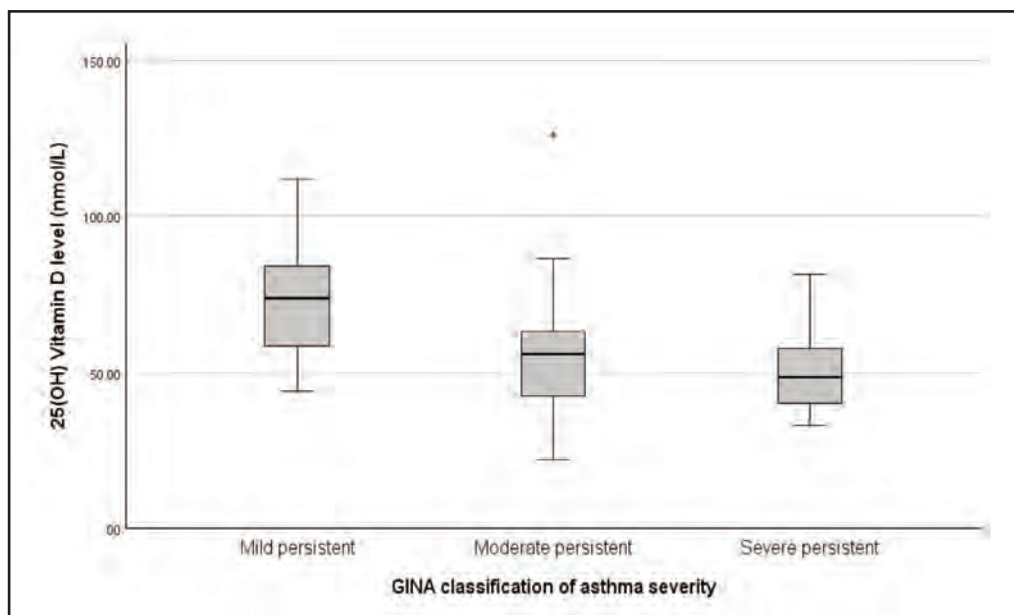


Fig. 2: The boxplot of serum vitamin D level in mild, moderate, and severe persistent asthma

among Muslims in Malaysia.<sup>15-17,24</sup> These practices can significantly reduce skin exposure to sunlight, thereby limiting the natural synthesis of vitamin D. Quah et al. found that being female, ethnicity of Malay and Indian, and consistently wearing long sleeves were associated with a higher likelihood of vitamin D deficiency.<sup>24</sup> Meanwhile, Anouti et al. from UAE demonstrated that over 80% of female migrants from Arab and South Asian countries were vitamin D deficient.<sup>25</sup>

Our study found that older children, particularly those in the adolescent age group have lower vitamin D levels. This observation aligns with a study by Al Sadat et al., which demonstrated a high prevalence of vitamin D deficiency among Malaysian adolescents, with 78.9% of those aged 12-13 years found to be deficient.<sup>16</sup> This finding could be linked to being adolescents often spend more time indoors due to academic commitments, increased use of electronic devices, and social activities which would limit their exposure to natural sunlight. Similarly, a study in Germany among children and adolescents found serum 25(OH)D levels generally decrease with age, starting high in younger age groups.<sup>26</sup> Additionally, hormonal changes during puberty in this age group, could also contribute to vitamin D deficiency, as these changes affect the body's metabolism and utilization of vitamin D.<sup>27</sup>

Those with higher BMI are found to have vitamin D deficiency, most likely due to lower physical activity and dietary intake affecting vitamin D metabolism.<sup>21,27</sup> A study conducted in Kuala Lumpur found a significant inverse association between vitamin D status and BMI-for-age among primary aged school children.<sup>28</sup> Similarly, a retrospective analysis among multi-ethnic Dutch children and adolescents found that vitamin D deficiency/insufficiency was highly prevalent among obese children.<sup>29</sup> This may be explained by the characteristics of

vitamin D itself as a fat-soluble vitamin; thus higher body fat reduces the availability of circulating serum 25(OH)D.<sup>21,27</sup>

The result of this study showed that vitamin D deficiency was significantly associated with increased asthma severity based on the GINA classification. Children with moderate and severe persistent asthma were more likely to have vitamin D deficiency than children with mild persistent asthma. The mean 25(OH)D level among our participants was 58.74 nmol/L, with lower levels correlating with higher asthma severity ( $p = 0.02$ ). This aligns with previous studies, by Sharif et al. which found that severe asthma cases in Iran had significantly lower vitamin D levels (53.4 nmol/L) compared to non-severe cases (70nmol/L).<sup>30</sup> In another study by Al-Zayadneh et al., among 98 asthmatic children, they found children with severe persistent asthma to have significantly lower vitamin D levels than those with intermittent asthma.<sup>13</sup> They also found deficient vitamin D group used significantly higher usage of systemic corticosteroids to treat asthma exacerbation within a year.<sup>13</sup> In a case control study in Turkey by Ozkars et al., the vitamin D levels in the mild and moderate persistent asthma groups were significantly lower than the non-asthmatic children.<sup>31</sup>

Vitamin D deficiency has been significantly associated with increased markers of asthma severity.<sup>32</sup> A study in Saudi by Aldubi et al. found that lower levels of vitamin D are linked to higher asthma prevalence and correlated with various inflammatory markers (interleukin-10, tumor necrosis factor-alpha, and platelet derived growth factor) and atopy markers (total IgE and eosinophil count).<sup>32</sup> Vitamin D has been shown to influence both innate and adaptive immune systems as well as airway cells, where its deficiency increases inflammation.<sup>10,33</sup> Studies indicate that its deficiency is associated with higher asthma risks, increased symptoms, more frequent exacerbations, and reduced lung function in those already suffering from asthma.<sup>10</sup> Furthermore, analyses

from birth cohorts showed that low maternal vitamin D intake and levels during pregnancy were linked to increased likelihood of wheezing in children at ages 3 and 5.<sup>33</sup> Unfortunately, we did not study the biomarkers of asthma severity among the children, and future research using these biomarkers is encouraged.

Our findings did not show the association between vitamin D levels and asthma control based on both assessment tools; ACT/c-ACT score and GINA classifications. This lack of statistically significant results was most likely due to asthma control could be influenced by many other confounding factors such as compliance to medications, environmental exposures, uncontrolled comorbid conditions, inhaled corticosteroid dosages and inhaler technique. Furthermore, our study population involved a small sample size in the uncontrolled group, as the majority of them (70.8 – 83.3%) had controlled asthma. Despite that, the trend of better asthma outcomes with sufficient vitamin D levels was seen, indicating that further investigation with larger sample sizes and diverse populations is needed to confirm these observations.

A case control study from Italy and Turkey found that lower vitamin D levels are associated with worse asthma control and poorer pulmonary function.<sup>23,34</sup> The other study, Esfandiar et al. however did not find an association between vitamin D deficiency and asthma control similar to our result.<sup>7</sup> Chinellato et al. otherwise found higher serum vitamin D levels moderately correlated with better asthma control, as indicated by c-ACT scores but not in the GINA group.<sup>34</sup> This variability in findings may be attributed to differences in study populations, sample sizes and methodologies. More comprehensive research is needed to establish a definitive association and understand the potential mechanisms through which vitamin D might influence asthma control.

Supplementation of vitamin D has been shown to improve asthma severity, enhance asthma control, and reduce exacerbations, suggesting its potential role as a potent immunomodulator in children with asthma.<sup>10,14,33</sup> A meta-analysis of 14 randomized controlled trials found that vitamin D supplementation was associated with a reduction in asthma exacerbation rates, reduced the risk of severe asthmatic symptoms and improved pulmonary function.<sup>35</sup> Additionally, a study among Japanese children with asthma demonstrated that low-dose vitamin D supplementation for two months significantly improved asthma control based on GINA classification and c-ACT scores.<sup>36</sup> This findings further highlight its potential benefit in asthma management.

The strength of our study was a sufficient sample size, which enhances the reliability and generalizability of the findings. However, our study has several limitations. Firstly, it is crucial to utilize objective methods to assess confounding factors related to vitamin D deficiency, such as sun exposure, dietary intake, and physical activity. A major challenge is that the existing research lacks clear definitions for adequate levels of these factors, which are significant determinants of vitamin D status. Objective tools, such as personal UV dosimeters to measure body sun exposure, accelerometers or pedometers to

track physical activity, particularly the time spent outdoors, could provide more accurate assessments. These devices otherwise could be costly and pose compliance challenges, particularly among the paediatric population. Furthermore, in our study, we were unable to quantify dietary intake of vitamin D, which represents a limitation to accurately assessing the contribution of diet to overall vitamin D levels. Future studies utilizing detailed dietary assessment questionnaires would provide a more comprehensive understanding of vitamin D intake and its impact on deficiency. Additionally, the study was conducted at a single centre, with all participants being Malay ethnicity, which may not be representative of the broader population of children with asthma, particularly those from diverse geographical or ethnic backgrounds.

## CONCLUSION

Our study highlights a high percentage of vitamin D deficiency and insufficiency among children with persistent asthma. There was a significant association between vitamin D deficiency and increased asthma severity. However, there was no significant association with asthma control. Factors associated with vitamin D deficiency are female, older age and higher BMI. Based on these study findings, we recommend routine assessments of vitamin D levels among children with moderate to severe asthma. This proactive approach may enable timely supplementation, potentially reducing asthma severity, improving control, lowering morbidity, and enhancing quality of life.

## ACKNOWLEDGEMENTS

We would like to thank the Director General of the Ministry of Health for the permission to publish this paper. We would like to extend our appreciation to the Department of Paediatrics, Hospital Raja Perempuan Zainab II, Kota Bharu, for their cooperation and support in carrying out this study and assisting with data collection. Finally, to all the parents and children at the Pediatric Clinic whom we had the great pleasure of meeting during my tenure there, we wish you all a blessed and happy life.

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# Artificial Intelligence (AI) in a Singaporean Emergency Department: Detecting fractures and reducing recalls

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## ABSTRACT

**Introduction:** There has been rapid increase in the number of artificial intelligence and machine learning (ML) algorithms in recent years. In our local emergency department (ED), after-hours, radiographs are read by the ED doctor, with formal reporting by the radiology department performed on the subsequent day. Discrepant diagnoses between the ED doctor and radiologist potentially result in recalls of discharged patients for additional treatment, leading to greater monetary and manpower costs. To the authors' knowledge, no Singapore based study has utilized local data to analyse the performance of an AI fracture detection solution in the Singapore ED. The objective of this study is to evaluate the diagnostic performance of an AI radiograph fracture tool compared to ED doctors.

**Materials and Methods:** A retrospective study was conducted on 42 discrepant radiographic studies. In these studies, the final radiology report by the radiology department (the "ground truth") had a different diagnosis from bedside radiographic assessment by an ED Doctor.

**Results:** There were 20 studies with fractures and 22 studies with no fractures. The AI solution correctly diagnosed 15 fractures (75.0% of cases with fracture) (Figure 1), missed 5 fractures (25.0% of cases with fracture) and overcalled 1 fracture (4.5% of cases with no fracture) (Figure 2). The AI solution sensitivity is 75.0%, specificity is 95.5%, positive predictive value (PPV) is 93.8% and the negative predictive value (NPV) is 80.8%.

**Conclusion:** Having a fracture detection AI solution has the potential of reducing discrepant cases by up to 73.7% in the ED setting. Further large-scale studies should be performed to quantify the economic, manpower and healthcare outcome benefits of such an AI solution.

## KEYWORDS:

*Artificial intelligence, discrepancy, fracture, radiograph*

## INTRODUCTION

There has been rapid increase in the number of artificial intelligence (AI) and machine learning (ML) algorithms in recent years due to advances in computational power and increase in legislative approval.<sup>1-14</sup> This is especially evident in radiology where AI has shown great potential to triage,

detect and classify abnormalities on imaging. Within the emergency department (ED), injuries to extremities account for up to 50% of non-fatal injuries, with almost all of these patients requiring radiographic imaging.<sup>15</sup> In our local ED, after-hours, radiographs are read by the ED senior physicians/consultants, with formal reporting by a radiology resident or consultant being performed only on the subsequent day. Discrepant diagnoses between the ED doctor and radiologist potentially result in recalls of discharged patients for additional treatment, leading to greater monetary and manpower costs on the healthcare system.<sup>16</sup> Apart from having 24 hours radiology radiographic reporting coverage which is labour intensive, some institutions have tried to decrease discrepancy rates by having monthly teaching/training meetings between the radiology and ED.<sup>17</sup> However, with the advancement in AI diagnostic algorithms, there exists now an additional method of increasing accuracy and efficiency in detecting and diagnosing fractures.<sup>1-5,7-9,12-14</sup> To the authors' knowledge, no Singapore based study has utilized local data to analyse the performance of an AI fracture detection solution in the Singapore ED. Moreover, while most AI articles focus on the diagnostic performance of AI in routine radiographs, this article focus on discrepant radiographs between ED physicians and radiologists, which are inherently more difficult.

## Aim

The objective of this study is to evaluate the diagnostic performance of an AI radiograph fracture detection support tool compared to radiographic assessment by emergency department doctors based on discrepant radiographs between ED physicians and radiologists.

## MATERIALS AND METHODS

A retrospective study was conducted on a set of discrepant radiographic studies collected over 3 consecutive months in 2019. These studies were from patients that were either 1) initially discharged from the ED without a diagnosis of fracture and later recalled for treatment following the diagnosis of fracture or 2) initially discharged from the ED with a diagnosis of fracture and later informed that no fracture was evident on radiograph. In these studies, the final radiology report by a radiology consultant or resident had a different diagnosis from bedside radiographic assessment by an ED Doctor. This retrospective study was approved by our institutional review board.

*This article was accepted: 23 June 2025*

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A total of 53 discrepant radiographic studies were acquired. Patient details and demographics were not recorded for the purpose of this study. Of these 53 discrepant radiographs, 11 studies were excluded from the study as these could not be processed by the AI solution – 4 were chest or spine radiographs and 7 were of skeletally immature patients.

The remaining 42 studies were processed by a commercial AI deep learning-based solution – RB Fracture (Radiobotics, Copenhagen, Denmark) on an AI orchestration platform - CARPL (CARPL.AI, US). RB fracture is an AI technology tool for pelvic and limb radiographs based on advanced computer vision and machine learning methods. It is E-marked as Class IIa according to (EU) MDR, commercially available AI solution that is FDA approved, and has been deployed in clinical settings in Europe and US. When a fracture has been detected, the AI solution will highlight the site of fracture with a square ROI (Figures 1 and 2).

All the 42 studies were also newly and independently reviewed by a pair of radiology consultants (one with 2 years and another with 7 years of specialty experience). The radiology consultants were blinded to the clinical history during assessment. If there was a disagreement between the two radiology consultants, the radiograph was to be reviewed by a third radiology consultant.

There were no financial or non-financial support by the vendors for this study. This study has been approved by the Institutional Review Board (IRB) (IRB registration 2023/2159).

### Ground Truth

The ground truth was defined as “fracture” or “no fracture” diagnosed on radiograph by both radiology consultants.

### RESULTS

Both the radiology consultants concurred with the initial radiologist for all 42 cases. Of the 42 discrepant radiographic studies, there were 20 studies with fractures and 22 studies with no fractures diagnosed by the pair of radiology consultants – “ground truth”. Radiographs with fractures only had one fracture each. All studies had 100% concordance between both radiology consultants.

The AI solution correctly diagnosed 15 fractures (75.0% of cases with fracture) (Figure 1), missed 5 fractures (25.0% of cases with fracture) and overcalled 1 fracture (4.5% of cases with no fracture) (Figure 2). The AI solution sensitivity is 75.0%, specificity is 95.5%, positive predictive value (PPV) is 93.8% and the negative predictive value (NPV) is 80.8%. For the 5 missed fractures, 4 fractures were located in the radial head of neck, 1 fracture was located in the talus. For the 1 fracture overcalled, the os naviculare was overcalled as a fracture.

### DISCUSSION

Globally, the EDs are facing increasing workload with ED attendance doubling in the past 20 years.<sup>7</sup> Locally, total ED attendance at public hospitals has grown approximately 5.57% per year between 2005 and 2016.<sup>18</sup> This results in an

increasing number of radiographic studies ordered in the ED and poses a great challenge to ED physicians, requiring them to read a greater volume of radiographs per ED shift.<sup>7,17,19</sup> However, compared to radiologists, ED physicians are at a disadvantage in reading such radiographic studies due to the utilization of non-dedicated diagnostic monitors, suboptimal room lighting, and significant time and patient turnaround pressures. Studies have reported a large variety of ED physician radiographic discrepancy rates ranging from 1.1%-23%.<sup>19-22</sup> Up to 24.2% of these patients were recalled back to the ED for further treatment.<sup>20</sup> The process of recalling is laborious and time intensive process which requires the patient to be seen a second time by the ED physician, incurring additional monetary and manpower costs. Recalling will also exacerbate overcrowding within the ED. Our study has shown that the AI solution can potentially reduce 75.0% recalls due to discrepant radiograph findings. In addition, the AI solution has a high specificity of 95.5% and PPV of 93.8%, implying that patients will unlikely undergo unnecessary fracture treatment. In a separate local study, the same AI solution has also proven to be effective in non-discrepant radiographs, with a sensitivity of 98.9%, an accuracy of 85.9%.<sup>23</sup>

Our study demonstrates a sensitivity of 75.0% likely because these were discrepant radiographs that were, by nature, more difficult radiographs that ED doctors and radiology trainees have misdiagnosed. On further analysis of the false negative cases (i.e fractures that were missed), majority of them were radial head/neck fractures which are notoriously difficult to diagnose.<sup>19,24</sup>

In the local Singapore context, AI fracture detection solutions also have the potential to reduce major discrepancy rates of radiology residents/trainees as a large proportion of hospital radiographs are formally reported by them. Notwithstanding reducing discrepancy rates, AI solutions have additional benefits of use in healthcare settings with limited resources. There is great potential to improve patient care by increasing diagnostic accuracy and reducing the burden on healthcare resources.<sup>3,5</sup> AI radiograph fracture detection support tools have all had significant published positive results, though they have different sensitivity and specificity depending on factors (definition of ground truth, the type of training and validation sets and the model function and algorithm). Some AI solutions have been shown to be superior in accuracy to radiology trainees (3), non-musculoskeletal trained radiologists (7), non-radiologists (2), and orthopaedic surgeons.<sup>14</sup> Kuo et al reported having a pooled sensitivity of 91% in fracture detection based on a meta-analysis of 37 studies which did not include RB fracture as an AI tool.<sup>5</sup> Guermazi et al. reported an increase in sensitivity of fracture detection by 10.4% and shortened average reading time by 6.3 seconds per radiograph.<sup>12</sup> Meynet et al. reported AI improving the sensitivity of radiologists for fracture detection by 20%, specificity by 0.6% and area under the ROC curve increased up to 10.6%.<sup>1</sup>

RB Fracture, the AI solution that was used in this study, has a reported relative reduction of missed fractures by the ED doctors of 43%.<sup>25</sup> The majority of published AI fracture detection tools focus on one anatomic area.<sup>15</sup> The major

Table I: Summary table of AI performance in all 42 radiographs

Site	Ground Truth - Fracture	AI Accuracy	Ground Truth.- No Fracture	AI Accuracy
Shoulder	3	3/3	1	1/1
Elbow	8	4/8	5	5/5
Pelvis	1	1/1	NA	NA
Femur	NA	NA	2	2/2
Knee	3	3/3	3	3/3
Ankle	4	3/4	7	7/7
Foot	NA	NA	4	3/4
Toes	1	1/1	NA	NA



Fig. 1: Left ankle radiograph in Mortise view. Left lateral malleolus fracture that was correctly diagnosed. The fracture site ROI was labelled by the AI fracture detection tool



Fig. 2: Right foot radiograph in Dorsal-Plantar view. Right os naviculare that was overcalled as a fracture. The "fracture" site ROI was labelled by the AI fracture detection tool

strength of RB Fracture is its ability to analyse multiple anatomic areas for fractures, reducing the need to have a separate solution for each region or body part.

Despite the positive findings of our study, we acknowledge some limitations and biases. Our analysis is limited by a small sample size. The retrospective assessment of these cases from an already categorized "discrepant case" poses selection biases in the analysis. We were unable to obtain additional cases for processing due to dataset issues and manpower exigencies during the study. Taking such a solution to full live implementation will require further testing on a larger local dataset with internal and external validation.<sup>3</sup> The "ground truth" in our study may also be subject to contention due to the human readers being blinded to clinical history. Extending this, some AI tools may be trained on such "subjective" ground truths resulting in selection and training biases.<sup>13</sup> In addition, currently RB fracture can only analyse pelvic and limb radiographs though this study has shown

great potential for future improvements to the algorithm. Regarding fractures, AI may be useful for majority of fractures but limited in pathological fractures which has great complications.<sup>14</sup> Needless to say, there remain outstanding ethical and legal issues and disclosures that require resolution and refinement before such solutions can be fully integrated into daily practice.<sup>3</sup>

Despite the present limitations, the future of AI Diagnostic solutions in the field of radiology is bright. Our study provides a good snapshot of AI's potential in enabling EDs to operate more safely and cost-effectively. Besides improving afterhours discrepancy rate, if the fracture detection tool is proven to be as accurate as radiology residents or non-subspecialised radiology consultant, ED physicians can potentially rely on AI during office hours and decide on disposition before the final report by the radiology resident or consultant. Increasing efficiency is key in the emergency department as it helps to minimize overcrowding. In addition to fracture

detection, there are other applications for AI in musculoskeletal trauma such as occult fracture prediction, diagnostic worklist prioritisation and image quality improvement.<sup>3,15</sup> These will be useful solutions that aid in the resource planning and prioritization/triage of appropriate care. With regards to AI fracture detection tools, few published studies at present have looked at human-algorithm performance through prospective clinical trials (5). Further studies should be undertaken to analyse large scale data prospectively to further ascertain the benefits and utility of having a fully capable AI solution for fracture detection.

## CONCLUSION

Having a fracture detection AI solution has the potential of reducing discrepant or recall cases in the ED by up to 75.0% while maintaining a high specificity of 95.5%. This is especially useful in times of reduced manpower hours for both radiologists and ED physicians. Whilst the results may vary across centres and depend on the exact type of AI solution deployed, the potential benefit of having a fracture detection AI solution in the ED setting is apparent.

Further large-scale studies should be performed to quantify the economic, manpower and healthcare outcome benefits of such an AI solution.

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# Psychometric validation of a healthcare professionals' attitude assessment scale toward maternal vaccination: A rasch model analysis

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## ABSTRACT

**Introduction:** Maternal vaccination is a proven strategy to protect newborns from vaccine-preventable diseases, yet its success depends heavily on healthcare professionals' attitudes and recommendations. In Malaysia, a validated and culturally appropriate tool to measure these attitudes is lacking. This study aimed to develop and validate a culturally relevant, reliable tool to assess healthcare professionals' attitudes toward maternal vaccination

**Materials and Methods:** The items were initially developed based on an extensive review of literature, vaccination guidelines, and expert reviews, followed by content and face validity involving nine and 30 reviewers, respectively. A pilot study was conducted on 196 respondents, and the reliability and validity were assessed using Rasch analysis (RA).

**Results:** Initially, 26 items were developed. However, only half of the items passed the content validity phase, with another two items removed post face validation. Eleven items were piloted for assessment of reliability and validity. Three items were removed due to concerns over their fit indices, and one item for redundancy. The final refined questionnaire consisted of seven items, demonstrating good reliability (person reliability = 0.80, separation index = 1.98), unidimensionality, and appropriate rating scale functioning, confirming its suitability for assessing healthcare professionals' attitudes toward maternal vaccination.

**Conclusion;** This study presents a psychometrically sound tool to measure healthcare professionals' attitudes toward maternal vaccination.

## KEYWORDS:

*Attitude, questionnaire, reliability, validity, maternal vaccination, rasch analysis*

## INTRODUCTION

Infections that are self-limiting in adults can potentially cause life-threatening problems to newborns due to immaturity of the immune system.<sup>1,2</sup> One example can be seen in pertussis, a highly contagious respiratory disease caused by the bacterium *Bordetella pertussis* which often presents as a mild upper respiratory tract infection in adults

but possess a significant risk to infants under two months of age.<sup>3</sup> Protecting infants from infection can be achieved through passive immunity via transplacental transfer of antibodies from mother.<sup>1,2</sup> Therefore maternal vaccination offers a tangible intervention, as they have been shown to reduce morbidity and mortality rates in both mothers and infants.<sup>4</sup>

The Ministry of Health Malaysia has taken an important step by integrating maternal vaccinations—such as tetanus-diphtheria-acellular pertussis (Tdap) and influenza vaccines—into the existing antenatal care framework. However, vaccine hesitancy remains a potential barrier to the success of this cost-effective public health intervention.<sup>4</sup> Healthcare professionals play a critical role in recommending and administering maternal vaccines, and their attitude have a profound influence on pregnant women's willingness to accept vaccination.<sup>5</sup> In settings where provider recommendation is a key determinant of vaccine uptake, understanding the attitudes of healthcare professionals is crucial for designing effective strategies to improve maternal immunisation coverage.<sup>6</sup> To facilitate this, there is a pressing need for a reliable, validated, and culturally appropriate instrument to accurately measure healthcare professionals' attitudes toward maternal vaccination, particularly in the Malaysian context where vaccine hesitancy may be shaped by diverse social and cultural factors.<sup>7</sup>

Traditional survey methods may not capture the perspectives of healthcare professionals accurately. By employing a robust psychometric analysis technique like Rasch model, a precise and reliable scale to measure latent traits, such as attitude can be developed.<sup>8</sup> In Malaysia, where maternal vaccinations are being considered for national implementation, no validated, culturally appropriate instrument currently exists to assess provider attitudes. Without such a tool, efforts to identify knowledge gaps, address hesitancy, or tailor interventions remain limited. Accordingly, this study aims to develop and validate a reliable questionnaire to assess the attitudes of healthcare professionals in Malaysia towards maternal vaccination uptake.

The development of this questionnaire was guided by the Theory of Planned Behaviour (TPB). According to TPB, an individual's intention to perform a behaviour is influenced by three key constructs: attitude toward the behaviour,

*This article was accepted: 08 July 2025*

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subjective norms (perceived social pressures), and perceived behavioural control (perceived ease or difficulty of performing the behaviour). In the context of maternal vaccination, healthcare providers' recommendations are shaped by their attitudes toward vaccines, their perception of expectations from peers and institutions, and their confidence in discussing or offering vaccines.<sup>9</sup> These constructs informed the generation and structuring of questionnaire items, ensuring theoretical alignment with the determinants of provider behaviour. The Rasch analysis was subsequently used to refine item performance and ensure unidimensionality of the attitude construct. However, this study only focuses on the attitude component and does not measure subjective norms or perceived behavioural control, as these aspects cannot yet be assessed since the vaccination program has not started.

## MATERIALS AND METHODS

This study employed a cross-sectional design to develop and validate a questionnaire assessing healthcare professionals' attitudes toward maternal vaccination.

### Stage 1: Item Generation

To inform item development, a structured literature search was conducted across PubMed, Cochrane Library, Epistemonikos, and CINAHL to identify existing instruments and conceptual domains related to healthcare professionals' attitudes toward maternal vaccination. Structured search strategy using Boolean operators (AND/OR) was used to combine population terms ("healthcare providers," "midwives," "nurses," "doctors"), construct terms ("attitude," "beliefs," "perception," "acceptance," "hesitancy"), intervention terms ("maternal vaccination," "antenatal vaccine," "pertussis vaccine," "influenza vaccine"), and instrument terms ("questionnaire," "survey," "scale," "tool," "psychometric validation"). The aim was to capture the multifaceted nature of attitudes as conceptualized by the TPB.

### Stage 2: Content and Face Validity

The preliminary items underwent content validation by a panel of five experts in public health, obstetrics, and psychometrics. Each expert independently assessed the relevance and clarity of each item using a 4-point Likert scale. The Content Validity Index (CVI) was calculated for each item, and items with a CVI below 0.78 were revised or removed, ensuring that the questionnaire items were both representative and comprehensible.<sup>9,10</sup> For face validity, the revised questionnaire was tested on 30 healthcare workers in Kelantan who care for pregnant women. Participants rated item clarity and comprehensibility using a four-point Likert scale, and written feedback was collected. Items with a face validity index (I-FVI) of at least 0.83 were retained in the final questionnaire.<sup>11</sup>

### Stage 3: Sampling and Data Collection

The finalised questionnaire was distributed to a convenience sample of 196 healthcare professionals who provide antenatal and maternal care across various healthcare settings, including government clinics, teaching hospitals, private general practices, and private hospitals in Kuantan,

Pahang. The sample encompassed a diverse group of professionals, such as midwives, nurses, medical officers, and obstetric specialists. Participation was voluntary, and informed consent was obtained from all respondents. The survey was conducted online using a self-administered questionnaire with no time constraints. The questionnaire consisted of two sections: sociodemographic and clinical background, and attitudes toward maternal vaccination.

### Stage 4: Rasch Analysis

Rasch analysis, performed using Winsteps 3.72.1, assessed reliability (person reliability and separation indices), validity (item fit, polarity, and discrimination index), item redundancy, and dimensionality.<sup>12</sup> The Wright Map was used to evaluate the scale's ability to differentiate between positive and negative attitudes toward maternal vaccination.<sup>13</sup>

Dimensionality was examined through principal component analysis (PCA) of residuals, comparing explained variance to unexplained variance from the first contrast. The rating scale's functioning was assessed based on response distribution, monotonicity, threshold calibration, category probability curves, and Outfit MNSQ statistics (14, 15). Table I summarizes the statistical criteria for reliability and validity using the Rasch Model.

## RESULTS

### Item generation

Through the literature review, we identified four key domains related to attitudes: vaccine efficacy, safety, necessity, and communication. These domains served as the foundation for our item development process. However, since no validated tool was available in Malaysia, we developed a new set of 22 items to comprehensively assess healthcare professionals' attitudes toward maternal vaccination.

### Sociodemographic and Clinical Experiences

We recruited 196 respondents in the pilot study. The mean age (standard deviation [sd]) were 40.5 (5.8) years with an average of 15.9 (6.3) years of working experience. The majority 71 (36.2%) were community nurses, followed by staff nurses 49 (25.0%), doctors 48 (24.5%) and midwives 28 (14.3%). Most respondents, 182 (92.9%) were working in government facilities. Educational levels varied, with 76 (38.8%) holding a Diploma or less and 13 (6.6%) having a postgraduate degree. Almost half of the respondents fell into the B40 income group (earning less than RM 5250.00 per month). 171 (87.2%) of respondents had experience administering anti-tetanus toxoid (ATT) to pregnant women. 180 (91.8%) had received influenza vaccine, with a small proportion reporting personal 20 (10.2%) and family 13 (6.6%) negative vaccination experiences. The characteristics of the respondents is presented in table II.

### Face and Content Validity

Content validity was evaluated by a panel of nine experts in infectious diseases, obstetrics, family medicine, and public health. The overall S-CVI was 0.83, indicating good content relevance. Of the 22 items, 14 (64%) were rated as relevant or highly relevant, achieving an I-CVI of at least 0.78. Eight items had lower I-CVI scores (0.4–0.7) and were excluded

**Table I: Criteria for reliability and validity using the Rasch Model**

Criteria	Statistics	Acceptable value
Reliability	Person reliability* Person separation*	> 0.63 > 1.25
Validity	Item polarity based on point measurement correlation (PT-MEA CORR) Item fit based on mean square infit and outfit value Discrimination	> 0.30 0.6 – 1.4 Close to 1.0
Unidimensionality assessed using principal component analysis (PCA) of the residuals.	Raw variance explained by the model Unexplained variance in the first contrast  First contrast disattenuated correlations	> 40% Less than 2.0 eigenvalue or less than 10.0% AND less than 1/3 of raw variance explained by items. > 0.6
Rating scale functionality assessment	Number per category, (d) distinct probability curve graph on each response category, and (e) Distribution of frequencies across categories  Monotonicity of average measures across rating scale categories  Spacing between step calibrations (thresholds)  Characteristics of Category Probability Curve (CPC)  Outfit MNSQ value of each category	> 10 per category  Regular distributions (i.e. uniform, normal, bimodal or slightly skewed) Trend of average measures should increase monotonically Threshold distances range from 1.4 to 5.0 logits, Each category should have a clear peak and distinct. 0.6 – 1.4

from face validation. The remaining 14 items were tested for face validity among healthcare workers involved in antenatal care in Kelantan. Three items had I-FVI scores below 0.83 and were removed, leaving 11 items in the final questionnaire.

#### Reliability and Validity

The questionnaire's reliability and validity were assessed using Rasch Analysis. Initially, the 11-item version showed a person reliability of 0.76 and a separation index of 1.77, but some items had fit and redundancy issues. Items 4, 6, and 11 had MNSQ values above 1.4, while items 9 and 10 showed high redundancy. After removing items 4, 6, 10, and 11, the refined 7-item version improved to 0.80 reliability and a 1.98 separation index, demonstrating good internal consistency and the ability to distinguish between two attitude levels. All remaining items had point-measure correlations above 0.30, MNSQ values within 0.6–1.4, and no redundancy (residual correlations <0.7), confirming a good fit to the Rasch model. Table III presents the full item analysis while the Wright map (Figure 1) visualised the position of items and persons based on their ability and difficulty respectively on the same scale.

#### Principal Component Analysis (PCA) and Dimensionality

The dimensionality of the questionnaire was assessed using Principal Component Analysis (PCA) of residuals. The PCA showed that the raw variance explained by the measures was 11.1 (61.3%), indicating that most of the variance was captured by the main construct. The unexplained variance in the first contrast was 1.8 eigenvalue, which was about one-third of the raw variance explained by the items (4.5 eigenvalue). This suggests that the questionnaire primarily measures a single construct, with no strong secondary dimension in the residuals, supporting its unidimensionality.<sup>12</sup>

#### Rating Scale Category Functioning

The rating scale's validity was assessed using Rasch analysis, focusing on key parameters. Each category had at least 10 responses, ensuring stable estimation. Responses were slightly skewed toward higher categories, reflecting a positive attitude trend. The average measures increased progressively, confirming monotonicity, while threshold calibration distances (1.4–5.0 logits) were within the acceptable range for category separation. Category probability curves showed distinct peaks, though minor overlap was observed between Categories 2 and 3, indicating some interpretability concerns. Outfit MNSQ values were mostly within the acceptable range, except for Category 1 (MNSQ = 1.75), suggesting potential misfit. Given the narrow threshold calibration distance between Categories 1 and 2, merging them would likely improve scale effectiveness. Figure 1 illustrates the Category Probability Curve (CPC).

#### DISCUSSION

Attitude is a strong determinant of intention and practice as explained by the theory of planned behaviour.<sup>16,17</sup> This causal relationship can be explained by the theory of planned behaviour by Ajzen and Fishbein, which states that attitude, along with 'perceived behavioural control' and 'subjective norm', are factors that shape the intention to behave in a certain way.<sup>18</sup> Healthcare professional with positive attitudes toward maternal vaccination are more likely to introduce and persuade their patients to get vaccinated (Gauld et al., 2022). In previous studies, recommendation from healthcare professionals have been found to be a major driving factors for maternal vaccine uptake.<sup>19,20</sup> This study is highly relevant in the context of Malaysia's ongoing efforts to strengthen maternal immunisation programmes, particularly with the anticipated national rollout of antenatal pertussis and influenza vaccines. As healthcare professionals are often the most trusted source of vaccine information for pregnant

Table II: Characteristics of Respondents in the Pilot Study

Variables	Frequency, n (%)
Age (years)	40.5 (5.8)*
Work Experience (years)	15.9 (6.3)*
Work position	196 (100.0)
Community Nurse	71 (36.2)
Staff nurse	49 (25.0)
Midwife	28 (14.3)
Medical Officer	39 (19.9)
Specialist	9 (4.6)
Workplace	196 (100.0)
Maternal and Child Health Clinic	154 (78.6)
Maternity clinic (Government Hospital)	25 (12.8)
Maternity clinic (Private Hospital)	8 (4.0)
General practices	9 (4.6)
Employer	196 (100.0)
Government	182 (92.9)
Private	11 (5.6)
Self-employed	3 (1.5)
Highest Level of Education	196 (100.0)
Diploma	76 (38.8)
Sarjana Muda (First degree)	39 (19.9)
Sarjana (Postgraduate)	13 (6.6)
Others	68 (34.7)
Household Income	196 (100.0)
< RM 5250.00	85 (43.4)
RM 5250 - 11,819	87 (44.4)
> RM 11,820	24 (12.2)
Experience Administering Anti-Tetanus Toxoid (ATT) to Pregnant Women	196 (100.0)
Yes	171 (87.2)
No	25 (12.8)
Has Taken Influenza Vaccine	196 (100.0)
Yes	180 (91.8)
No	16 (8.2)
Personal Bad Vaccination Experience	196 (100.0)
Yes	20 (10.2)
No	176 (89.8)
Bad Vaccination Experience in Family	196 (100.0)
Yes	13 (6.6)
No	183 (93.4)

\* mean (sd)

Table III: Rasch analysis of items in the attitude questionnaire

Item	Infit		Outfit		PT-MEA CORR	Discrim.
	MNSQ	ZSTD	MNSQ	ZSTD		
1	0.86	-1.2	0.82	-1.5	0.80	1.22
2	0.69	-2.9	0.75	-1.1	0.75	1.27
3	0.80	-1.8	0.78	-1.6	0.78	1.16
5	1.12	1.0	0.64	-0.4	0.64	0.96
7	1.22	1.9	1.21	1.8	0.78	0.81
8	0.81	-1.7	0.76	-2.1	0.82	1.23
9	1.40	3.4	1.59	4.4	0.73	0.43
Mean (SD)	0.99 (0.24)	-0.2 (2.1)	0.98 (0.29)	-0.1 (2.2)	0.76 (0.06)	1.01 (0.31)

MNSQ = mean square, ZSTD = Z-standard score, PT-MEA CORR = Point measurement correlation, Discrim = discrimination index

women, accurately assessing their attitudes is critical for designing effective interventions, training programmes, and policy responses aimed at improving maternal vaccine uptake.

In this study, we employed RA due to its superior diagnostic ability in validating and assessing the reliability of the developed questionnaire, rather than the commonly employed, CTT.<sup>8</sup> In RA, each item is treated individually,

where the probability of response to an item is independent from responses to other items and has equal contribution to the total score (additive conjoint measurement).<sup>12</sup> This attribute allows us to examine each item in detail including how they function across different levels of attitude and determine its fit with the overall scale. Problematic items can be removed or modified accordingly.<sup>12</sup> RA also produces invariant measurement, where the estimation of item difficulty is independent of the estimation of respondents'



Our findings are consistent with previous validation studies on provider attitude tools, which also demonstrated the value of Rasch-based refinements in improving measurement precision. Unlike many earlier tools that were adapted cross-culturally without thorough psychometric testing, this questionnaire was developed from the ground up for a specific cultural and healthcare context.

A more robust assessment of validity can be achieved with a larger sample size and more random sampling. Despite using non-randomised sampling, the inclusion of healthcare providers from diverse clinical roles and hierarchies from both public and private sectors, improve the representativeness of the sample. The approach also provides a replicable framework for other low- and middle-income countries (LMICs) aiming to develop culturally grounded tools for assessing healthcare worker perceptions—an often overlooked but critical component of implementation science. Furthermore, by using Malay language and validated through Rasch analysis, the questionnaire holds promise for generalisability in Malaysia healthcare setting and potentially across other Southeast Asian countries with comparable healthcare systems and sociocultural contexts.

While person misfit analysis is a standard step in RA, we prioritised ensuring scale robustness across a diverse respondent pool. Removing misfitting individuals could lead to a loss of real-world variations in healthcare professionals' attitudes.<sup>22,23</sup> Instead, we focused on evaluating item fit, unidimensionality, and rating scale validity to confirm the tool's psychometric properties. Given the nature of attitude measurement, variations in responses are expected and do not necessarily indicate data errors. Additionally, given our sample size of 196, removing participants would have further reduced statistical power, potentially leading to unstable item estimates. While sensitivity analysis was not performed, the overall Rasch fit statistics confirmed that the refined scale functions reliably, supporting our decision to retain all respondents for analysis.<sup>23</sup>

Although the PCA indicated that the first contrast explained only 9.8% of the variance with an eigenvalue of less than 2.0, the subsequent contrasts explain progressively smaller portions of variance with decreasing eigenvalues (1.3, 1.2, 1.0, and 0.8). This pattern suggests an absence of strong secondary dimensions.<sup>24</sup> The unidimensionality of the scale was further supported by the fit of each item to the Rasch model. All seven items in the final scale had infit and outfit MNSQ values between 0.6 and 1.4 where values below 0.6 indicate overfit (predictable responses) and values above 1.4 indicate underfit.<sup>8</sup> Additionally, the polarity of all items which indicates the correlation between the item's score and the overall measure (or trait) being assessed, were positive and had values of more than 0.3.<sup>12</sup> All these findings confirm the unidimensionality of the questionnaire.

The Wright Map findings confirm that the scale effectively differentiates healthcare professionals based on their attitudes toward maternal vaccination. While the item distribution does not cover extreme attitude levels, this is not a limitation given the study's objectives.<sup>25</sup> The primary goal of this scale is not to finely rank respondents across multiple

strata but to broadly classify them into key attitude groups (e.g., favourable vs. less favourable attitudes). Since this is not a high-stakes examination or competency test, such differentiation is sufficient for identifying general trends and informing public health interventions.<sup>25</sup> This makes the tool particularly useful for programmatic use by public health agencies, where the focus is more on identifying priority groups for training and support rather than precise measurement of individual scores.

Future work should consider longitudinal testing of the tool's responsiveness to change following educational interventions or policy shifts. Further cross-cultural validation in multilingual populations, particularly in regions with similar vaccine rollout plans, could expand its utility. Additionally, linking attitude scores with actual provider behaviours (e.g., vaccine recommendation rates) would further establish the scale's predictive validity. The scale provides a practical and meaningful classification, ensuring its applicability in real-world public health settings where targeted interventions are needed rather than precise ranking of individuals.

#### LIMITATIONS

This study used a non-random, convenience sampling method, which may limit the generalizability of the findings. While efforts were made to include participants from diverse professional backgrounds and healthcare settings, the lack of random sampling introduces the potential for selection bias. Therefore, the attitudes captured by this tool may not fully represent all healthcare professionals involved in maternal care across Malaysia. Future studies should consider employing random sampling strategies with larger sample sizes to further validate the scale and enhance its external validity.

#### CONCLUSIONS

Since healthcare workers' attitude towards maternal vaccination plays an important determining factor for a successful vaccination program, a valid tool that can accurately measure attitude is important. The newly developed questionnaire demonstrates good psychometric properties with acceptable reliability, allowing for an accurate assessment of the attitude. Through Rasch analysis, a questionnaire with unidimensional construct capable of discriminating those with negative and positive attitudes was produced. Despite the used of non-randomised sampling and a relatively small number of respondents, we were able to achieve a high overall item reliability and a good degree of generalisability. Future study with larger sample size and more diverse respondents could further improve the validity of this questionnaire.

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# High-fidelity simulation vs video-assisted teaching for early electrocardiography learning - Randomised controlled trial

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## ABSTRACT

**Introduction:** Electrocardiography (ECG) interpretation is a vital yet challenging competency for preclinical medical students due to limited clinical exposure. While high-fidelity simulation teaching (HFST) offers immersive, experiential learning, video-assisted teaching (VAT) provides scalable, consistent instruction. Despite growing interest in both methods, comparative evidence remains limited. This study aimed to evaluate the effectiveness of HFST versus VAT in enhancing ECG knowledge and retention among preclinical students using a Multiple-Choice Question (MCQ) assessment for knowledge cognition.

**Materials and Methods:** It was a randomised controlled trial study where 136 first year undergraduate medical students were randomised into control (VAT) and intervention (HFST) groups. The intervention group received a 20-minute simulation session using SimMan, demonstrating ECG lead placement, interpretation, and basic management, followed by a 20-minute interactive discussion whereas the control group underwent a VAT session mirroring the HFST content with a 20-minute pre-recorded video followed by a 20-minute faculty-led discussion. MCQs were used to assess the acquisition and retention of knowledge, both as a pre-test (week 1) and a post-test (week 12). The outcomes were measured using the mean and standard deviation of the total scores of MCQ. Intragroup analyses were conducted using a dependent sample t-test, whereas intergroup analyses were performed using an independent samples t-test. ANCOVA was used to assess the difference in post-test MCQ scores among the groups after adjusting the Pre-test scores. The P-value was set at 0.05. "Institutional research trial registration number": RMC\_NUIR\_2024\_21

**Results:** Both groups demonstrated significant enhancement in knowledge gain and retention from the pre-test to the post-test. The VAT group showed a greater mean score improvement compared to the HFST group. While the VAT group outperformed the HFST group in the post-test, this difference was not statistically significant. Adjusting for pre-test scores using ANCOVA further confirmed the non-significance of post-test score differences between the two groups. Two-way mixed ANOVA revealed no significant interaction between group and time point, indicating comparable patterns of knowledge gain across both teaching methods.

**Conclusion:** Both HFST and VAT significantly increased knowledge and retention among preclinical medical students. The VAT group showed a slightly higher knowledge gain than the HFST group, although the difference was statistically insignificant. The study demonstrated that both VAT and HFST were effective in mid-term knowledge acquisition and may offer a viable alternative for inclusion in undergraduate preclinical curricula.

## KEYWORDS:

High-fidelity Simulation, simulation-based medical education, video-assisted learning, MCQ, RCT

## INTRODUCTION

Knowledge of Electrocardiography (ECG) interpretation is an essential competency in medical education that remains a challenging topic for students across various healthcare professions. Accurate interpretation of ECG readings is not merely about memorizing patterns; it demands a deep understanding of how to situate these findings within relevant clinical contexts.<sup>1</sup> This presents a formidable challenge for preclinical medical students, who frequently lack sufficient exposure to real-life cases and practical experiences.<sup>2</sup>

Most medical schools have included ECG-related physiology and pathology in their course curricula, which start in the first year, with learning continuing into the clinical years. However, multiple studies have reported that students often lack the confidence and competence necessary to interpret ECGs during their formative training years accurately.<sup>3,4</sup> While senior medical students may demonstrate adequate proficiency in interpreting basic ECG parameters, they frequently struggle with recognizing complex patterns and emergencies.<sup>5</sup> Consequently, this early gap in training can undermine their confidence, hinder their performance, and foster the belief that mastering ECG-related concepts is an insurmountable task.<sup>2</sup>

In response to this problem, various instructional strategies have been contemplated to enhance ECG teaching. These include blended learning approaches that combine face-to-face instruction with online modules, standalone web-based tutorials and flipped classroom models incorporating team-

This article was accepted: 08 July 2025

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based learning.<sup>2,6-10</sup> These techniques have been reported to enhance learning outcomes due to factors such as increased student autonomy, flexible access to resources, and better conceptual understanding with real-time scaffolding. Despite these innovations, there remains no consensus on the most effective instructional method, particularly concerning long-term retention and clinical applicability in the early preclinical year's perspective.

Simulation-based teaching has gained prominence as an innovative and immersive approach without exposing patients to the associated risks. It helps bridge the gap between theoretical learning and real-world clinical experience.<sup>11</sup> High-fidelity simulation (HFS), which utilizes computer-driven models to replicate clinical scenarios, is increasingly recognized for its ability to foster cognitive skills, clinical reasoning, teamwork, and professional behaviour.<sup>12</sup> It has also been shown to boost student satisfaction and engagement, particularly in settings with limited access to clinical sites or teaching faculty.<sup>13</sup> Furthermore, simulation may augment emotional investment, promoting memory retention, and is believed to prepare students more effectively for patient interactions.<sup>14-16</sup> Evidence supports the efficacy of HFS in delivering immediate knowledge gains and improving long-term retention. A study by Maddry et al. found that although lecture-based instruction was initially effective, students taught via simulation retained information better three months after the intervention.<sup>17</sup> Kashou et al. found web-based self-directed learning to be beneficial.<sup>18</sup> Whereas other RCTs involving flipped classrooms or algorithm-based instruction produced mixed outcomes, citing concerns over instructional clarity and learner adaptation.<sup>19,20</sup> These findings suggest the need for teaching methods tailored to both curricular goals and learner characteristics. Most existing literature focuses on blended or augmented teaching strategies rather than direct comparisons between simulation and standalone video methods. Additionally, recent findings emphasise the use of simulation to reinforce critical decision-making under realistic constraints, which is particularly valuable in ECG interpretation training.<sup>21,22</sup> Video-assisted teaching (VAT) has gained traction due to its scalability and adaptability, which also facilitate the consistent delivery of complex content and allow learners to control the pace of their education.<sup>23</sup> Despite the growing popularity of HFS, its application in ECG instruction for early-stage medical students remains underexplored, particularly through well-designed randomized controlled trials (RCTs). However, while HFS has become more widely used, RCTs directly comparing its efficacy with other methods in ECG education remain limited. Further high-quality evidence is required to validate its benefits and guide curriculum development.<sup>24</sup> In this context, our study aimed to assess the efficacy of high-fidelity simulation as compared to traditional video-assisted lectures in teaching ECG to preclinical medical students. The findings aimed to inform evidence-based curricular decisions and identify the most effective approaches for teaching complex diagnostic skills, such as ECG interpretation.

## MATERIALS AND METHODS

### Study Design

Randomized Controlled Trial (RCT) study with parallel

groups and 1:1 allocation. Please see Figure 1 for the Flow Chart.

### Recruitment and Eligibility Criteria

A total of 144 first year MBBS students in the institute (male and female) were recruited after obtaining their written informed consent. The students who declined consent were excluded from the study. The participants were between the ages of 18-21 years. Ultimately, 136 participants completed the study. The number of participants who dropped out of the study was eight (6.25%). The study was conducted between October 2024 and April 2025 (a period of seven months) in the Simulation Lab and Control Room [H.1.4] of Newcastle University Medicine, Malaysia.

### Interventions

The participants who were offered VAT were designated as the control group (CG). The intervention group (IG) was provided with high-fidelity simulation teaching (HFST).

Description of Video Assisted Teaching (VAT) for the control group: It was based on a 20-minute pre-recorded video clip demonstrating a simulated case scenario that included ECG lead placements on the mannequin, interpretation of ECG findings, and basic management. It was taught by a faculty who was involved in the delivery of a preclinical curriculum for the MBBS programme at NUMed Malaysia. The video-assisted teaching was immediately followed by an interactive discussion session with the faculty for another 20 minutes.

Description of HFS-based teaching for the Intervention Group: A 20-minute facilitated simulation session identical to that of CG. It was demonstrated on SimMan by another faculty member who was involved in the delivery of a preclinical curriculum for the MBBS program at NUMed Malaysia. The hands-on training session was followed by an interactive discussion session for another 20 minutes.

Description of Hi-fidelity simulator: SimMan Hi-Fidelity Simulator (3G SN: 21247123132) was used for the simulation sessions. It is an advanced emergency care patient simulator designed for high-fidelity simulated scenarios, offering a fully immersive experience. Simulated complex medical cases can be created that enable learners to prepare for real-world situations. It helps learners practice critical skills, such as decision-making and team communication, for developing high-quality patient care.

### Outcomes:

All participants appeared for the Pre-test and Post-test knowledge assessments in the first and twelfth weeks. Identical single best answer multiple choice questions (MCQ) were used in both the Pre-test and Post-test for assessing the gain and retention of knowledge.

The usefulness of HFST and VAT methods was assessed by noting the difference in the Pre-test and Post-test scores of the two groups. MCQs were constructed based on the learning outcomes of the interventions.

### Sample Size

G\* software and data from the pilot study were used for the calculation of the sample size.<sup>25</sup> The value of two independent means for continuous data was estimated by means of 80%

power and 5% type I error. A moderate effect size of 0.44 was accepted based on the pilot study. The sample size thus calculated was 128. 10% was added over it for allowance of dropouts.

### Randomisation Process

A computer-generated random sequence method by utilising randomizer.org, was used to randomly assign the participants into control (VAT) and intervention (HFST) groups. A block size of two was applied. An independent member of the research team was involved in the enrolment process. The randomization process was assigned to a biostatistician who was not involved in delivering the interventions. While another member was involved in assigning the participants to their respective rooms for the delivery of the interventions. The outcome assessor and the biostatistician were blinded to the group allocation.

### Data Collection

For each session, a cohort of 12 to 14 was recruited. Initially, a briefing was given to the participants regarding the learning outcomes, training course sessions, and the assessment protocol. All of them were informed about the modality of the teaching interventions, confidentiality, and the ethical issues involved. An introduction to the HFS SimMan was made for all participants in the simulation lab to inform them of its functionalities, including the handling of the equipment. They were assured that the training course was not part of the evaluation process for their first-year course curriculum. After the briefing session, all the participants appeared for the first knowledge assessment (Pre-test MCQ) for evaluation of their initial background knowledge about ECG physiology and ECG interpretations of common cardiac pathologies (myocardial ischemia, infarction, hypertrophy, cardiac arrhythmias, heart blocks) that had already been taught as part of the first-year course curriculum. The randomization process followed the Pre-test. Immediately after the briefing session, the participants were randomized into control (VAT) and intervention (HFST) groups. There were 6 to 7 participants in each group after the randomization process. The control group then participated in the VAT session, whereas the intervention group participated in a facilitated HFST session on SimMan. The total duration of both interventions (VAT and HFST) was 40 minutes. There were no provisions for additional teaching sessions for either the VAT or HFST groups during the course of the study. The facilitators involved in delivering the interventions were informed about the importance of maintaining uniformity in the teaching sessions to achieve optimal learning outcomes.

In the 12th week, both VAT and HFST groups appeared for the second knowledge assessment (Post-test MCQ) to assess their gain and retention of knowledge. The Post-test MCQ assessment was delayed, reducing recall bias and providing a better measure of knowledge retention.

Both the Pre-test and Post-test assessments consisted of 20 single-best-answer (A-type) multiple-choice questions (MCQs) to be completed within 20 minutes. The MCQs were designed in accordance with the guidelines of the National Board of Medical Examiners.<sup>26</sup> Each correct answer would receive one point, with no negative marking for incorrect responses.

Based on the defined learning objectives, the MCQs were developed by six subject experts in the fields of Pathology, Physiology, Medicine, and Medical Education, all of whom were not part of the research team. The questions focused on ECG-related aspects of physiology, pathology, and interpretation. The MCQs were designed to assess knowledge comprehension, clinical reasoning, and application. Identical MCQs were used for both Pre-test and Post-test assessments. To minimize recall bias, the order of questions was adjusted between the Pre-test and Post-test assessments.

A pilot study involving 36 students was conducted to explore the time management, feasibility, acceptability, and validation of the multiple-choice questions (MCQs). This includes evaluating item difficulty using the difficulty index and item discrimination using the point bi-serial correlation. MCQs with a difficulty index between 20 and 80 and a bi-serial correlation of 0 to 0.14 were considered acceptable for this study. The Optical Mark Recognition (OMR) answer sheets were scanned and analysed using software provided by the Speedwell system (<https://www.speedwellsoftware.com>).

The students in the VAT group were provided with access to the same high-fidelity simulation sessions at the end of the course to ensure parity in their development of knowledge. Likewise, the HFST group was also given access to VAT at the end of the course. The students who had not consented to the research study were also provided with access to both teaching modalities.

### Statistical Analysis

The data was analysed using IBM SPSS software (version 29). Descriptive analysis was used to describe the characteristics of the respondents, including frequency and percentage for categorical data, as well as the overall mean score of the knowledge assessments. An independent samples t-test was used to compare the mean scores of MCQs among the VAT and HFST groups, whereas a dependent samples t-test was carried out for intragroup comparisons of pre and post-knowledge assessments. The difference in post-test MCQ scores between the VAT and HFST groups was calculated using the principle of Analysis of Covariance (ANCOVA) with Pre-test MCQ scores as covariates. The effect size (partial eta squared) was calculated using ANCOVA to compare VAT and HFST Post-test MCQ scores. Cohen's *d* was calculated for the intragroup comparison of Pre-test and Post-test MCQ scores. The difference in MCQ scores between the control and intervention groups over assessment time points was calculated using a two-way mixed ANOVA. All the statistical tests were two-sided, and the level of significance (*p*-value) was set at 0.05.

Institutional research trial registration number: RMC\_NUIR\_2024\_21(12th July 2024)

Ethical registration number: 2900/49590 (27th September 2024)

### RESULTS

A total of 136 preclinical medical students were included in the study, with equal allocation to VAT and HFST groups (Table I). The overall gender distribution showed a higher

**Table I: Baseline demographic information of the participants**

	Groups	Gender		Median Age (years)		Type of students		Total
		Male	Female	Local	International	Local	International	
Enrolled	VAT	28	44	19.1	20.3	36	36	144
	HFST	27	45			32	40	
Dropped out	VAT	3	1			2	2	8
	HFST	2	2			3	1	
Completed	VAT	25	43			34	34	136
	HFST	25	43			29	39	

**Table II: Intragroup comparison of pre and post MCQ scores among intervention and control groups. (Dependent samples t-test)**

Variable (groups)	n	Mean (SD)		Mean difference (95% CI)	t (df)	p-value	Dz
		Pre-test score	Post-test score				
Control (VAT)	68	8.40 (2.55)	9.65 (3.03)	1.25 (-1.96, -0.54)	-3.525 (67)	< 0.001*	0.428
Intervention (HFST)	68	8.28 (3.33)	9.19 (3.59)	0.91 (-1.73, -0.09)	-2.227 (67)	< 0.001*	0.270

\* Significant  
 n: number of participants  
 SD: Standard Deviation  
 CI: Confidence Interval  
 Dz: Cohen's dz

**Table III: Intergroup comparison of pre and post MCQ scores among intervention and control groups. (Independent samples t-test)**

Variable (groups)	n	Mean (SD)		Mean difference (95% CI)	t (df)	p-value	Dz
		Control (VAT)	Intervention (HFST)				
Pre-test score	68	8.40 (2.55)	8.28 (3.33)	0.12 (-0.89, 1.12)	0.232 (125)	0.817	0.04
Post-test score	68	9.65 (3.03)	9.19 (3.59)	0.46 (-0.67, 1.58)	0.800 (134)	0.425	0.14

n: number of participants  
 SD: Standard Deviation  
 CI: Confidence Interval  
 Dz: Cohen's dz

**Table IV: Intergroup comparison of pre and post MCQ scores among intervention and control groups with Pre-test MCQ scores as covariate (ANCOVA).**

Variable (groups)	n	Post-test MCQ score		Mean difference	Observed Power <sup>b</sup>	p-value	Partial Eta Squared
		Mean <sup>a</sup>	Standard Error				
Control (VAT)	68	9.614	0.350	0.39	0.122	0.433	0.005
Intervention (HFST)	68	9.224	0.350				

a. Covariates appearing in the table were evaluated at the following values: Pretest Score = 8.3382.  
 b. Computed using alpha = 0.05

proportion of females (63.2%) compared to males (36.8%), with identical proportions observed in both study arms. In terms of nationality, the majority of participants were international students (53.7%), compared to Malaysians (46.3%). The HFST group comprised 57.3% international students compared to 42.7% local students, while the VAT group had an equal distribution of international and local students.

Table II illustrates within-group comparisons of Pre-test and Post-test scores. Overall, both groups exhibited a significantly higher gain and retention of knowledge. The VAT group showed greater improvement than the HFST group in terms of mean score difference. The effect size was moderate in the VAT group (Cohen's dz = 0.428). The effect size for the HFST group was small (Cohen's dz = 0.270).

Table III presents the comparison of knowledge gain and retention between the control and the intervention groups. At baseline, the mean score for the VAT group was slightly higher compared to the intervention group. However, this difference was not statistically significant (p = 0.817), indicating comparable baseline knowledge among the participants. In the post-test, the VAT group performed better than the HFST group; however, the difference remained statistically non-significant (p = 0.425). It suggested that neither instructional method (VAT and HFST) had yielded a superior outcome than the other in terms of post-intervention performance. The effect size was small in both groups.

The VAT group showed better performance than the HFST group in the Post-test knowledge assessment. However, after adjusting the pre-test MCQ scores as a covariate, the

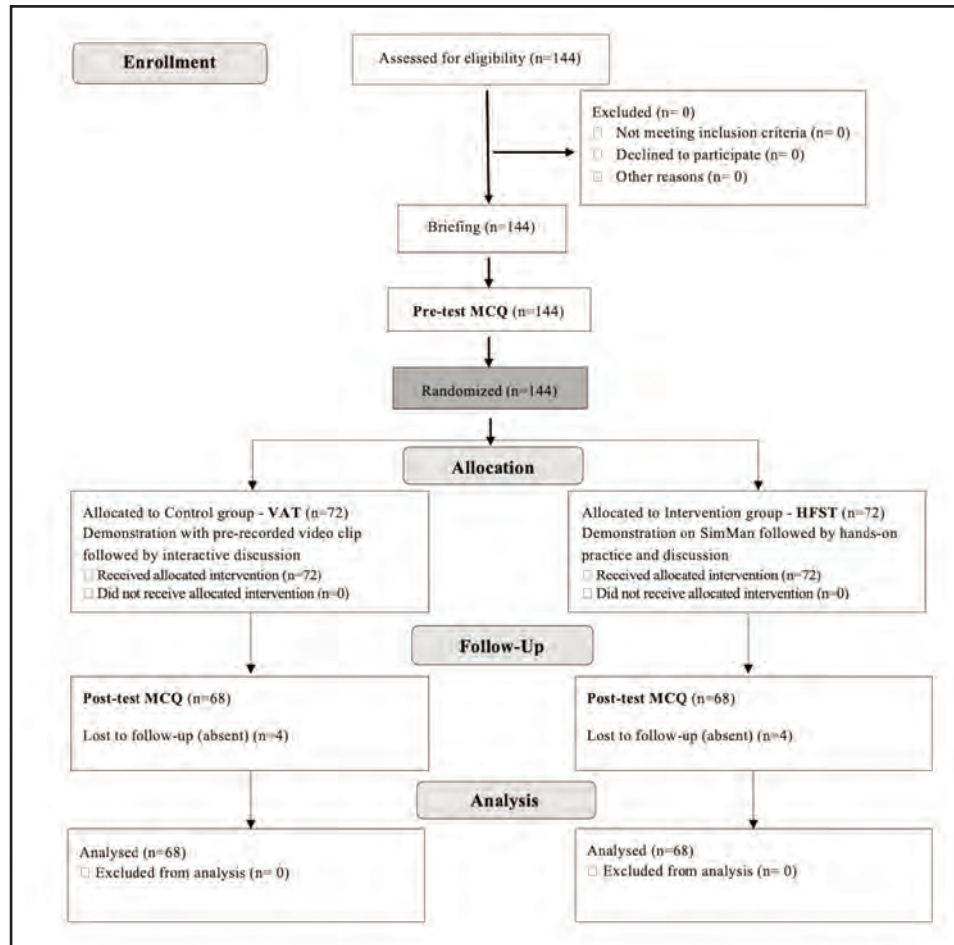


Fig. 1: Flowchart of participant recruitment, intervention, and results.

difference in post-test MCQ scores between the groups was statistically insignificant ( $p = 0.433$ ). The effect size was minimal (See Table IV).

A two-way mixed ANOVA was conducted to evaluate differences in MCQ scores between the VAT and HFST groups across different assessment time points. There was no significant interaction between the type of intervention and time points on MCQ scores was found, indicating similar patterns of knowledge change in both groups. The assumption of homogeneity of variances was satisfied, as evidenced by Levene's test, which pointed no significant difference ( $p = 0.410$ ).

The data of the study is available at <https://doi.org/10.25405/data.ncl.27600783>

## DISCUSSION

Traditional teaching methods, such as didactic lectures and static visual aids, often struggle to provide the interactive and experiential learning crucial for mastering essential skills. Video-assisted teaching (VAT) uses multimedia for consistent and accessible instruction, allowing students to learn independently.<sup>6</sup> While VAT can improve short-term memory, it often fails to foster deep understanding and real-world

application of ECG knowledge.<sup>1</sup> Additionally, passive learning methods may not effectively engage students or simulate the dynamic environments they will encounter in practice.<sup>27</sup> With the limitations of traditional methods, there's a shift toward active, learner-centered approaches like high-fidelity simulation teaching (HFST). This transformative approach offers immersive, hands-on experiences replicating real clinical scenarios. Research has demonstrated that these simulations significantly enhance clinical confidence and deepen understanding of complex concepts.<sup>28</sup> In this context, the study aimed to explore the efficacy of two different teaching modalities in the knowledge domain, specifically in mid-term retention of knowledge, based on the positive trends observed in the study's results. The importance of innovative teaching strategies in medical education is being explored worldwide, whereas previous analysis suggested such a combination could maximize learning efficiency and retention.<sup>1,6</sup>

Our study revealed better outcomes in terms of knowledge gain and retention in both teaching modalities. This corroborates the findings of a systematic study performed by Cheng et al., which demonstrated that simulation-based education not only enhances knowledge acquisition but also increases learner confidence, satisfaction, and long-term skill retention.<sup>29</sup> Recent studies have explored the impact of

simulation on ECG education. For instance, Zheng et al. found that integrating high-fidelity simulation into cardiovascular physiology increased student engagement, response times, and understanding of ECG principles.<sup>30</sup> The participants who were exposed to HFS teaching scored higher in the Post-test knowledge assessment that reinforces the suitability of HFS inclusion in the preclinical course curriculum.<sup>31</sup>

Mixed learning approaches that combine traditional lectures with interactive elements have been shown to improve understanding and retention of ECG skills.<sup>27</sup> Asynchronous digital platforms with video modules and quizzes have proven to be as effective as conventional lectures for teaching ECG concepts.<sup>32</sup> These methods are associated with increased learner confidence and better retention of ECG skills.<sup>1,6</sup> For example, Viljoen et al. reported that blended learning improved competence and confidence in ECG among medical students compared to traditional methods.<sup>2</sup> Similarly, Olvet and Sadigh highlighted that asynchronous e-modules were as effective as lectures teaching ECG interpretation to first-year students.<sup>6,27</sup>

Conventional teaching modalities help enhance knowledge in some learners, but additional simulation teaching may support greater knowledge gain and overall satisfaction in preclinical medical education.<sup>33</sup> We compared two different teaching interventions that aligned with the findings of the above research studies. While both groups improved, the VAT group demonstrated a larger mean difference than the HFST group, with a higher level of statistical significance and a larger effect size. The VAT group performed better than the HFST group in both Pre-test and Post-test knowledge assessments, although the difference was statistically insignificant. This contradicted the findings of previous studies and the assumption that HFST is superior to VAT.<sup>21,34</sup><sup>38</sup> Our study also highlighted that VAT has a greater impact on knowledge acquisition, which also supported the findings of Alluri et al.<sup>38</sup> Studies directly comparing HFST and VAT were sparse, particularly in the domain of ECG education. Existing evidence suggests that while HFST may excel in skill acquisition and contextual learning, VAT is advantageous for reinforcing theoretical knowledge and enabling flexible, repeated access to material, leading to better outcomes.<sup>39</sup> In ECG instruction, video resources can standardize teaching across diverse student cohorts, ensuring all learners receive equivalent exposure to core concepts, and that may explain the better performance of the VAT group as compared to the HFST group.<sup>2</sup>

This study was able to effectively randomize and allocate students to further stringent the analysis on comparing the effectiveness of HFST and VAT through the MCQ test. There was a similar gender distribution in both the intervention and control groups, hence minimizing gender as the confounding variable in the comparison of the MCQ outcomes. Alongside, both groups had equal sample sizes, which strengthened the statistical power and validity of the results.

## STUDY LIMITATIONS

Despite its robust design, this study acknowledged several limitations. First, the generalizability of the findings may be limited due to the single institution setting and small sample size, which could potentially underrepresent diverse student demographics. There is a possibility of selection bias due to the randomization in block size of two, where the allocation of the participants may become predictable. The likelihood of bias could be expected due to the different teaching faculty delivering the interventions in VAT and HFST. This might have influenced the delivery of the content due to variance in teaching styles, ultimately impacting the outcomes of the study. Additionally, the analysis did not control for the baseline academic performance prior to exposure to content or the learning style of students, which could potentially impact the results. Moreover, confounding variables such as discussions between students from different groups, recall memory, and preparation for the Post-test knowledge assessment could potentially influence the results. Further studies could expand on these limitations by including multi-centre studies, longer follow-ups, and additional outcome measures like clinical performance or cost-effectiveness.

## CONCLUSION

HFST and VAT demonstrate a similar impact on enhancing the knowledge and retention of pre-clinical students. At baseline, both groups demonstrated equivalent levels of knowledge, with no statistically significant difference. Hence, validating the difference in performance measured later would be due to the teaching approaches rather than any existing gaps. Both HFST and VAT groups showed improvements in knowledge gain and retention. However, the post-intervention analysis showed that the VAT group had performed slightly better than the HFST group. This difference was not statistically significant, indicating that neither teaching method was significantly superior for mid-term knowledge acquisition nor retention. Both teaching approaches appeared to be equally effective in conveying the relevant content. Moreover, with the widespread use of HFST, VAT also demonstrated a higher efficacy as an educational approach for certain learning objectives. Further studies are necessary to compare the cost effectiveness and resource requirements of HFST and VAT in determining the potential benefits for practical implementation. Moreover, long-term outcomes and effectiveness of various teaching methods, adjusting for different variables to identify the most effective approaches in medical education also needs to be explored.

## ACKNOWLEDGEMENT

We would like to acknowledge Newcastle University Medicine Malaysia for the invaluable support. We would like to express our sincere gratitude to Prof. Joanna Matthan (Dean of Academic Affairs) for her guidance and constant encouragement, which played a vital role in shaping the direction and quality of this research.

We also wish to convey our heartfelt appreciation to the NUMed teaching fellows—Dr. Pavitraa Annandan, Dr. Daniel Chiam, and Dr. Arvin Thurairaj—for their unwavering support and instrumental contributions.

We are equally grateful to all the students who participated in the research. Their enthusiasm, cooperation, and willingness to engage meaningfully made this project possible.

#### DECLARATION OF INTEREST

The authors declare that they have not received any funding or benefits to conduct this study and have no conflicts of interest.

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# Impact of challenging-to-treat areas on quality of life and mental health among plaque psoriasis patients from Sabah, Malaysia

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## ABSTRACT

**Introduction:** Patients with psoriasis with involvement of challenging-to-treat-areas often have higher disease burden. We studied the impact of the challenging-to-treat areas namely scalp, face, nail, palms, soles and genitalia on the quality of life and mental health of psoriatic patients.

**Materials and Methods:** We conducted a cross-sectional study involving 120 patients aged 18 years and above with plaque psoriasis. Those with challenging-to-treat-areas were compared to those without.

**Results:** The prevalence of challenging-to-treat-areas were scalp (35.8%), face (26.7%), nails (38.3%), palms (6.7%), soles (5.0%) and genitalia (15.0%). Psoriasis Area and Severity Index (PASI) was higher in patients with challenging-to-treat areas, with a median of 4.55 (IQR: 3.00, 7.80) compared to 1.80 (IQR: 0.60, 2.85) in those without ( $p < 0.001$ ). Patients with these areas demonstrated a significantly higher Disease Life Quality Index (DLQI) scores compared to those without (mean  $\pm$  SD: 5.13  $\pm$  6.17 vs 11.02  $\pm$  7.19;  $p < 0.001$ ). Overall the prevalence of depression, anxiety and stress, scored using Depression, Anxiety and Stress Scale-21 Item (DASS-21), were 19.2%, 28.3% and 14.2% respectively with two thirds of patients had challenging-to-treat areas. Significantly more patients with challenging-to-treat-areas suffered from moderate to extremely severe depression (8.4% vs 5.0%,  $p = 0.034$ ) and anxiety (33.4% vs 16.7%,  $p = 0.001$ ). Patients with challenging-to-treat areas had higher mean itch Numeric Rating Scale (NRS) (4.28  $\pm$  2.90) compared to those without (1.45  $\pm$  1.58) ( $p < 0.001$ ).

**Conclusion:** Patients with psoriasis with challenging-to-treat-areas reported greater impairment in quality of life and higher risk of depression and anxiety.

## KEYWORDS:

Challenging-to-treat, psoriasis, DLQI, mental health, itch NRS

## INTRODUCTION

Psoriasis is a chronic, immune-mediated inflammatory disease that is associated with social stigmatization, physical discomfort and psychosocial burdens, that significantly

reduce patients' quality of life (QOL) and mental health status.<sup>1-5</sup> The involvement of challenging-to-treat areas such as scalp, face, nails, palms, soles and genitalia have been noted to cause much distress and affecting patients' QOL though often small in size, can cause substantial distress and significantly affect QOL.

Patients with scalp psoriasis are usually concern of the high visibility and persistent itch.<sup>2,4,6</sup> Due to visibility, small area of involvement the face can affect one's emotional well-being and confidence. Nail psoriasis can be painful, resulting in decreased work productivity.<sup>2,5,7</sup> Palmoplantar disease can be itchy and painful thus causing functional impairment.<sup>2,8</sup> Other than these, genital involvement which is frequently overlooked during clinical assessments, can cause itch, burning and embarrassment which interfere with patients' daily lives and sexual relationships.<sup>9</sup>

Standard measurements of disease severity such as body surface area (BSA) involvement or plaque severity may not effectively capture the psychological distress caused by the involvement of these areas, when even small areas have significant impact.<sup>1,2,4</sup> These areas are also challenging-to-treat with topical therapy alone and may require systemic treatments, which aren't always effective.<sup>2,4,5</sup> Furthermore, patients often seek quick relief, presenting challenges for physicians in managing unrealistic expectations.<sup>5</sup>

Currently, there is no consensus on the best treatment for these areas,<sup>2,4,5</sup> with existing studies mainly focusing on Western demographics and QOL, while mental health risks remain underexplored. In Eastern contexts like Malaysia, psoriasis impacts may vary due to differences in disease awareness, societal perceptions, and treatment access. Understanding socio-demographics, clinical characteristics, disease activity, and mental health is essential for developing effective treatment strategies.<sup>2,4,5</sup>

This study aims primarily to assess the impact of challenging-to-treat areas on QOL and mental health in plaque psoriasis patients at Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia. It will additionally explore socio-demographic and clinical characteristics, and evaluate the relationship between disease involvement and itch, QOL, depression, anxiety, and stress.

This article was accepted: 08 July 2025

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## MATERIALS AND METHODS

### Study Type and Design

This is a cross-sectional, descriptive study conducted on plaque psoriasis patients at the Dermatology Clinic, Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia, involving 120 patients from 1st September 2023 to 31st March 2024. Inclusion criteria were patients aged 18 or older with plaque psoriasis. Exclusion criteria included patients under 18, those with other types of psoriasis; e.g., guttate, erythrodermic, pustular psoriasis, and those with severe concurrent medical or dermatological conditions; e.g., active malignancy, dementia, or systemic diseases like SLE or dermatomyositis.

Approval from the Medical Research and Ethics Committee (MREC) of the Ministry of Health (MOH) Malaysia was obtained prior to initiation of the study. The study was conducted in compliance with ethical principles outlined in the Declaration of Helsinki and Malaysian Good Clinical Practice guidelines.

Informed consent was obtained from all subjects. Data were collected on the case report form. These included demographics, medical history, clinical assessments using Psoriasis Area and Severity Index (PASI), Physician Global Assessment (PGA) for the scalp, face, palms, soles and genitalia and for the nail, the Nail Psoriasis Severity Index (NAPSI) scoring, and treatment received for the past 6 months.

The PASI assessment is done by dividing the body into four regions; legs (40%); trunk (30%); arms (20%); and head (10%). Each region's surface area is measured as a percentage and scored from 0 to 6 (0=1%; 1=<10%; 2=10-29%; 3=30-49%; 4= 50-69%; 5=70-89%; 6=90-100%). Severity is evaluated using three parameters: erythema (E), infiltration (I) and desquamation (D). These are evaluated on a scale of 0 to 4, from none to 'very severe' (None=0; Some=1; Moderate=2; Severe=3; Very Severe =4). Each region's severity scores are added, multiplied by the area score, and then by the percentage of skin in that region; Head:  $(I + E + D) \times A \times 0.1 = \text{Total head}$ ; Arms:  $(I + E + D) \times A \times 0.2 = \text{Total arms}$ ; Body:  $(I + E + D) \times A \times 0.3 = \text{Total body}$ ; Legs:  $(I + E + D) \times A \times 0.4 = \text{Total legs}$ . The PASI score ranges from 0 to 72, with higher scores indicating more severe disease. Interpretation can be categorised into < 8 is mild, 8 to 12 is moderate and >12 is severe.<sup>10</sup>

The PGA scoring evaluates three components namely erythema, induration, and scaling. The components are assessed separately for all psoriatic lesions and scored on a scale from 0 to 4. The severity scores are summed, and the average score is calculated, then rounded to the nearest integer to determine the final PGA score. The final PGA score ranges 0 to 4, where 0 represents "Clear," 1 indicates "Almost Clear," 2 corresponds to "Mild," 3 denotes "Moderate," and 4 represents "Severe".<sup>11</sup>

The NAPSI score assesses the severity of nail involvement. Imaginary horizontal and longitudinal lines are used to divide the nail into four quadrants. Each nail is given a score for nail bed psoriasis (0-4) and nail matrix psoriasis (0-4)

depending on specific features of nail psoriasis in that quadrant. Nail matrix psoriasis is evaluated by presence of any pitting, leukonychia, red spots in the lunula or/ and crumbling in each quadrant and will be scored with: 0 for none, 1 if any or all features are present in 1 quadrant of the nail, 2 if present in 2 quadrants of the nail, 3 if present in 3 quadrants of the nail, and 4 if present in 4 quadrants of the nail. Nail bed psoriasis is evaluated by presence of any onycholysis, splinter haemorrhages, subungual hyperkeratosis and "oil drop" (salmon patch dyschromia) and will be scored with: 0 for none, 1 for 1 quadrant involvement of any or all the features mentioned, 2 for 2 quadrants, 3 for 3 quadrants, and 4 for 4 quadrants. Each nail receives a matrix score and a nail bed score, the sum of both scores is the score for that nail (0-8, e.g.: nail matrix score of 2 and nail bed score of 1 give a total score of 3) and the total NAPSI score ranges 0-160 as this study includes the scoring of both the fingernails and toenails.<sup>12,13</sup>

The patients then complete a set of questionnaires survey including the scorings of Itch Numeric Rating Scale (NRS), Dermatology Life Quality Index (DLQI) and Depression, Anxiety and Stress Scale-21 Item (DASS-21). The questionnaires were available in English and Bahasa Malaysia.

The Itch NRS is a single-item, patient-reported outcome (PRO) designed questionnaire to assess itch severity. Patients rate their itch severity in the past 24 hours on an 11-point scale with 0 representing 'no itch' and 10 representing 'the worst itch imaginable'. It is interpreted as: NRS 0 means no pruritus; NRS < 3 is mild pruritus; NRS  $\geq 3 - < 7$  is moderate pruritus; NRS  $\geq 7 < 9$  is severe pruritus and NRS  $\geq 9$  is very severe pruritus.<sup>14</sup>

The DLQI is a self-administered questionnaire which measures the health-related QOL of adults with skin diseases. It consists of 10 questions on the impact of skin diseases on various aspects of life over the past week. Scores range from 0 to 3 per question, with interpretations as follows: 0 – 1, no effect at all on patient's life; 2–5, small effect on patient's life; 6–10, moderate effect on patient's life; 11–20, very large effect on patient's life; and 21 – 30, extremely large effect on patient's life.<sup>15-18</sup>

The DASS-21 is a set of three self-report scales assessing depression, anxiety, and stress, each with 7 items. The depression scale assesses dysphoria, hopelessness, devaluation of life, self-deprecation, lack of interest or involvement, anhedonia and inertia. The anxiety scale assesses autonomic arousal, skeletal muscle effects, situational anxiety, and subjective experience of anxious affect. The stress scale assesses difficulty relaxing, nervous arousal, and being easily upset or agitated, irritable or over-reactive and impatient. Scores are summed up for each category then multiplied by 2 to calculate the final score. Scores are interpreted as in Table I.<sup>19-22</sup>

### Statistical Analysis

The data were analysed using SPSS version 26.0. Continuous variables were assessed for distribution using skewness, kurtosis, and histograms, and presented as mean  $\pm$  standard

deviation (normal distribution) or median with interquartile range (IQR) (non-normal distribution). Categorical variables were presented as frequency and percentage.

Differences between plaque psoriasis patients with and without challenging-to-treat areas were analysed using Independent Sample t-test, Mann Whitney U test, Pearson chi-squared test, and Fisher Exact test. Spearman correlation was used to explore the relationship between severity of lesions, itch, QOL and mental health of the patients, due to the ordinal nature and /or skewed distribution of the variables. The relationships were further tested using Fisher Exact test using the categorize form of the variables. All tests were two sided and statistical significance was defined as  $p < 0.05$ .

## RESULTS

### Demographic characteristics of the patients

Table II illustrates the demographic characteristics of the patients. A total of 120 patients were recruited with a mean age of 46 years old (SD = 15.55). The mean duration of psoriasis was 11.68 years (SD: 10.85), with 67 patients (55.8%) being diagnosed within the past year.

Those with challenging-to-treat areas were younger, with a mean age of 44.02 years (SD = 14.09), though the difference was not statistically significant ( $p=0.091$ ). The gender distribution was fairly balanced with 52.5% females and 47.5% males with no significant differences between the two groups. The sample consisted of native Sabahans (80.9%), followed by Chinese (14.2%), Malay (4.2%) and Indians (0.8%).

The mean BMI was 28.64 kg/m<sup>2</sup> (SD = 5.79) with a higher prevalence of overweight or obesity among patients with challenging-to-treat areas (78.0%). These patients also had higher mean BMI (29.80 kg/m<sup>2</sup>, SD = 6.28) compared to those without (27.49 kg/m<sup>2</sup>, SD = 5.04), although a small difference, it was statistically significant ( $p=0.030$ ). Other comorbidities included hypertension (35.8%), dyslipidaemia (30.8%), diabetes (15.8%), chronic kidney disease (7.5%), cardiac disease (4.2%), chronic liver disease (0.8). There were 11 patients (9.2%) reported to be alcoholic, 19 (15.8%) smoke and 7 (5.8%) vape.

### Clinical characteristics of the patients

Table III highlights the clinical characteristics of the patients, comparing those with and without challenging-to-treat areas. The PASI score was significantly higher in patients with challenging-to-treat areas, with a median of 4.55 (IQR: 3.00, 7.80) compared to 1.80 (IQR: 0.60, 2.85) in those without ( $p < 0.001$ ). This is further reflected in the PASI category, where a higher proportion of patients with challenging-to-treat areas had severe psoriasis (16.7%) compared to those without (1.7%) ( $p=0.001$ ).

The challenging-to-treat areas included scalp (35.8%), face (26.7%), nail (38.3%), palm (6.7%), soles (5.0%) and genitalia (15.0%), with severity ranging from clear to moderate. For patients with nail involvement, the median NAPSI score was 17.5 (IQR: 1.5, 37.25).

In terms of treatment, topical therapy was used by majority of the patients (98.3%), while 61 patients (50.8%) received systemic therapy and 4 patients (3.3%) underwent phototherapy.

The mean itch NRS score was significantly higher for patients with challenging-to-treat areas ( $4.28 \pm 2.90$ ) compared to those without ( $1.45 \pm 1.58$ ) ( $p < 0.001$ ). This difference was also evident in the itch NRS category, where 23.3% of patients with challenging-to-treat areas reported severe or very severe itch compared to none in the group without ( $p < 0.001$ ).

The DLQI score was worse in patients with challenging-to-treat areas, with a higher mean score of  $11.02 \pm 7.19$  compared to  $5.13 \pm 6.17$  in the group without ( $p < 0.001$ ). A higher percentage of patients with challenging-to-treat areas reported moderate to extremely large effects on their QOL (78.3%) compared to the group without (36.7%) ( $p < 0.001$ ).

Overall, 19.2%, 28.3% and 14.2% of the patients reported depression, anxiety and stress respectively. Two thirds of them had involvement of challenging-to-treat areas. Comparing to those without, significantly more patients with challenging-to-treat areas had higher DASS-21 score indicating moderate to extremely depression (5.0% vs 8.4%  $p=0.034$ ) and moderate to extremely severe anxiety (16.7% vs 33.4%,  $p = 0.001$ ).

### Relationship between severity of challenging-to-treat areas with quality of life, itch and mental health

Table IV shows the relationship between severity of lesions at challenging-to-treat areas with quality of life, mental health and itch.

The scalp severity was significantly positively correlated with DLQI scores (coefficient: 0.487;  $p < 0.001$ ) and a positive correlation was observed between scalp severity with DASS-21 total scores (coefficient: 0.302;  $p= 0.001$ ), DASS-21 depression scores (coefficient: 0.268;  $p= 0.003$ ) and DASS-21 stress scores (coefficient: 0.362;  $p < 0.001$ ).

A significant positive correlation was observed between face severity and DLQI scores (coefficient: 0.344;  $p < 0.001$ ) as well as DASS-21 stress scores (coefficient: 0.184;  $p=0.044$ ).

In terms of palm and sole severity, only DLQI scores showed a significant positive correlation, with palm severity (coefficient: 0.253;  $p = 0.005$ ) and sole severity (coefficient: 0.250;  $p= 0.006$ ).

A significant positive correlation was observed between genitalia severity with DLQI scores (coefficient: 0.341;  $p < 0.001$ ), DASS-21 total scores (coefficient: 0.254;  $p=0.005$ ), DASS-21 depression scores (coefficient: 0.240;  $p=0.008$ ) and DASS-21 stress scores (coefficient 0.320;  $p < 0.001$ ).

Furthermore, a significant positive correlation was observed between NAPSI scores and DLQI scores (coefficient: 0.367;  $p < 0.001$ ), DASS-21 total scores (coefficient: 0.253;  $p=0.005$ ), DASS-21 depression scores (coefficient: 0.249;  $p=0.006$ ) and DASS-21 stress scores (coefficient: 0.256;  $p = 0.005$ ).

Table I: Score interpretation of DASS-21

	Depression	Anxiety	Stress
Normal	0-9	0-7	0-14
Mild	10-13	8-9	15-18
Moderate	14-20	10-14	19-25
Severe	21-27	15-19	26-33
Extremely Severe	28+	20+	34+

Table II: Demographic characteristics of the patients

Variables		Overall	Without challenging-to-treat area	With challenging-With to-treat area	P value
Age in years, mean ± SD		46.42 ± 15.55	48.82 ± 16.66	44.02 ± 14.09	0.091 <sup>a</sup>
Gender, n (%)	Female	63 (52.5)	31 (51.7)	32 (53.3)	0.855 <sup>b</sup>
	Male	57 (47.5)	29 (48.3)	28 (46.7)	
Race, n (%)	Malay	5 (4.2)	0 (0.0)	5 (8.3)	0.532 <sup>c</sup>
	Chinese	17 (14.2)	10 (16.7)	7 (11.7)	
	Indian	1 (0.8)	1 (1.7)	0 (0.0)	
	Kadazan	24 (20.0)	13 (21.7)	11 (18.3)	
	Dusun	23 (19.2)	11 (18.3)	12 (20.0)	
	Bajau	13 (10.8)	7 (11.7)	6 (10.0)	
	Rungus	9 (7.5)	5 (8.3)	4 (6.7)	
	Sabahan Brunei	8 (6.7)	4 (6.7)	4 (6.7)	
	Other Sabah ethnicities	20 (16.7)	9 (15.0)	11 (18.3)	
Employment status, n (%)	Unemployed / Retired	44 (36.7)	25 (41.7)	19 (31.7)	
	Employed	76 (63.3)	35 (58.3)	41 (68.3)	
Educational level, n (%)	No formal schooling	3 (2.5)	1 (1.7)	2 (3.3)	0.170 <sup>c</sup>
	Primary	13 (10.8)	7 (11.9)	6 (10.0)	
	Secondary	49 (40.8)	19 (32.2)	30 (50.0)	
	Tertiary	54 (45.0)	32 (54.2)	22 (36.7)	
Household income, n (%)	<RM3490 (low)	65 (54.2)	30 (50.0)	35 (58.3)	0.617 <sup>b</sup>
	RM3490-RM8199 (medium)	43 (35.8)	24 (40.0)	19 (31.7)	
	≥RM8200 (high)	12 (10.0)	6 (10.0)	6 (10.0)	
Marital status, n (%)	Not married	32 (26.7)	16 (27.1)	16 (27.1)	>0.950 <sup>b</sup>
	Married	86 (71.7)	43 (72.9)	43 (72.9)	
BMI in kg/m <sup>2</sup> , mean ± SD		28.64 ± 5.79	27.49 ± 5.04	29.80 ± 6.28	0.030 <sup>a</sup>
Overweight / Obese, n (%)	No	34 (28.3)	21 (35.6)	13 (22.0)	0.104 <sup>b</sup>
	Yes	84 (70.0)	38 (64.4)	46 (78.0)	
Duration of psoriasis, mean ± SD		11.68 ± 10.85	12.99 ± 11.86	10.37 ± 9.66	0.187 <sup>a</sup>
Duration from onset to diagnosis of disease within a year, n (%)	No	52 (43.3)	26 (43.3)	26 (43.3)	>0.950 <sup>b</sup>
	Yes	68 (56.7)	34 (56.7)	34 (56.7)	
Comorbidities, n (%)	Hypertension	43 (35.8)	20 (33.3)	23 (61.7)	0.568 <sup>b</sup>
	Diabetes Mellitus	19 (15.8)	7 (11.7)	12 (20.0)	0.211 <sup>b</sup>
	Dyslipidaemia	37 (30.8)	18 (30.0)	19 (31.7)	>0.950 <sup>b</sup>
	Chronic kidney disease	9 (7.5)	5 (8.3)	4 (6.7)	>0.950 <sup>c</sup>
	Cardiac disease	5 (4.2)	4 (6.7)	1 (1.7)	0.364 <sup>c</sup>
	Chronic liver disease	1 (0.8)	1 (1.7)	0 (0.0)	>0.950 <sup>c</sup>
Alcohol intake, n (%)	No	109 (90.8)	54 (90.0)	55 (91.7)	0.752 <sup>b</sup>
	Yes	11 (9.2)	6 (10.0)	5 (8.3)	
Smoking, n (%)	No	101 (84.2)	51 (85.0)	50 (83.3)	0.803 <sup>b</sup>
	Yes	19 (15.8)	9 (15.0)	10 (16.7)	
Vaping, n (%)	No	113 (94.2)	58 (96.7)	55 (91.7)	0.439 <sup>c</sup>
	Yes	7 (5.8)	2 (3.3)	5 (8.3)	

<sup>a</sup>Independent sample t test; <sup>b</sup>Pearson chi-squared test; <sup>c</sup>Fisher Exact test

The itch severity was found to be correlated with all the QOL and mental health measures. A positive correlation was observed between itch NRS and DLQI (Coefficient: 0.547;  $p < 0.001$ ), DASS-21 total scores (coefficient: 0.366;  $p < 0.001$ ), DASS-21 depression scores (coefficient: 0.313;  $p < 0.001$ ), DASS-21 anxiety scores (coefficient: 0.223;  $p=0.014$ ) and DASS-21 stress scores (coefficient: 0.376;  $p < 0.001$ ).

#### Association between severity of challenging-to-treat areas with quality of life

A significant association was observed between the DLQI category with scalp severity ( $p < 0.001$ ), face severity ( $p = 0.001$ ), and genitalia severity ( $p = 0.008$ ) (Table V).

Table III: Clinical characteristics, Itch NRS, DLQI and DASS-21 scores

Variables		Overall	Without challenging-to-treat area	With challenging-to-treat area	p value
PASI score, median (IQR)		2.95 (0.90, 5.55)	1.80 (0.60, 2.85)	4.55 (3.00, 7.80)	<0.001 <sup>b</sup>
PASI category, n (%)	Mild	106 (88.3)	59 (98.3)	47 (78.3)	0.001 <sup>d</sup>
	Moderate	3 (2.5)	0 (0.0)	3 (5.0)	
	Severe	11 (9.2)	1 (1.7)	10 (16.7)	
Psoriatic arthritis, n (%)	No	101 (84.2)	49 (81.7)	52 (86.7)	0.453 <sup>c</sup>
	Yes	19 (15.8)	11 (18.3)	8 (13.3)	
Involvement of challenging-to-treat area, n (%)	Scalp	43 (35.8)	NA	43 (71.7)	NA
	Face	32 (26.7)		32 (53.3)	
	Nail	46 (38.3)		46 (76.7)	
	Palm	8 (6.7)		8 (13.3)	
	Sole	6 (5.0)		6 (10.0)	
	Genitalia	18 (15.0)		18 (30.0)	
Scalp severity, n (%)	Clear	77 (64.2)	NA	17 (28.3)	NA
	Almost clear	28 (23.3)		28 (46.7)	
	Mild	14 (11.7)		14 (23.3)	
	Moderate	1 (0.8)		1 (1.7)	
Face severity, n (%)	Clear	88 (73.3)	NA	28 (46.7)	NA
	Almost clear	25 (20.8)		25 (41.7)	
	Mild	7 (5.8)		7 (11.7)	
Palm severity, n (%)	Clear	112 (93.3)	NA	52 (86.7)	NA
	Almost clear	6 (5.0)		6 (10.0)	
	Mild	2 (1.7)		2 (3.3)	
Soles severity, n (%)	Clear	114 (95.0)	NA	54 (90.0)	NA
	Almost clear	4 (3.3)		4 (6.7)	
	Mild	2 (1.7)		2 (3.3)	
Genitalia severity, n (%)	Clear	102 (85.0)	NA	42 (70.0)	NA
	Almost clear	15 (12.5)		15 (25.0)	
	Mild	3 (2.5)		3 (5.0)	
NAPSI, median (IQR)		0.00 (0.00, 17.75)		17.50 (1.50, 37.25)	
Topical therapy, n (%)	No	2 (1.7)	2 (3.3)	0 (0.0)	0.496 <sup>d</sup>
	Yes	118 (98.3)	58 (96.7)	60 (100.0)	
Phototherapy, n (%)	No	116 (96.7)	57 (95.0)	59 (98.3)	0.619 <sup>d</sup>
	Yes	4 (3.3)	3 (5.0)	1 (1.7)	
Systemic therapy, n (%)	No	59 (49.2)	27 (45.0)	32 (53.3)	0.361 <sup>c</sup>
	Yes	61 (50.8)	33 (55.0)	28 (46.7)	
Itch NRS, mean ± SD		2.87 ± 2.73	1.45 ± 1.58	4.28 ± 2.90	<0.001 <sup>a</sup>
Itch NRS category, n (%)	None	33 (27.5)	22 (36.7)	11 (18.3)	<0.001 <sup>c</sup>
	Mild	43 (35.8)	29 (48.3)	14 (23.3)	
	Moderate	27 (22.5)	9 (15.0)	18 (30.0)	
	Severe	14 (11.7)	0 (0.0)	14 (23.3)	
	Very severe	3 (2.5)	0 (0.0)	3 (5.0)	
DLQI, mean ± SD		8.07 ± 7.30	5.13 ± 6.17	11.02 ± 7.19	<0.001 <sup>a</sup>
DLQI category, n (%)	No effect	28 (23.3)	22 (36.7)	6 (10.0)	<0.001 <sup>c</sup>
	Small effect	25 (20.8)	18 (30.0)	7 (11.7)	
	Moderate effect	28 (23.3)	9 (15.0)	19 (31.7)	
	Very large effect	28 (23.3)	8 (13.3)	20 (33.3)	
	Extremely large effect	11 (9.2)	3 (5.0)	8 (13.3)	
DASS-21 total score, median (IQR)		12 (2, 28)	21 (6, 36)	32 (18, 42)	<0.001 <sup>b</sup>
DASS-21 depression, median (IQR)		2 (0, 8)	0 (0, 4)	4 (0, 10)	0.001 <sup>b</sup>
DASS-21 depression category, n (%)	Normal	97 (80.8)	54 (90.0)	43 (71.7)	0.034 <sup>d</sup>
	Mild	15 (12.5)	3 (5.0)	12 (20.0)	
	Moderate	6 (5.0)	3 (5.0)	3 (5.0)	
	Severe	1 (0.8)	0 (0.0)	1 (1.7)	
	Extremely severe	1 (0.8)	0 (0.0)	1 (1.7)	
DASS-21 anxiety, median (IQR)		4 (0, 10)	3 (0, 6)	4 (2, 10)	0.023 <sup>b</sup>
DASS-21 anxiety category, n (%)	Normal	86 (71.7)	50 (83.3)	36 (60.0)	0.001 <sup>d</sup>
	Mild	4 (3.3)	0 (0.0)	4 (6.7)	
	Moderate	21 (17.5)	6 (10.0)	15 (25.0)	
	Severe	5 (4.2)	4 (6.7)	1 (1.7)	
	Extremely severe	4 (3.3)	0 (0.0)	4 (6.7)	
DASS-21 stress, median (IQR)		6 (0, 12)	2 (0, 6)	8 (4, 14)	<0.001 <sup>b</sup>
DASS-21 stress category, n (%)	Normal	103 (85.8)	55 (91.7)	48 (80.0)	0.215 <sup>d</sup>
	Mild	10 (8.3)	4 (6.7)	6 (10.0)	
	Moderate	3 (2.5)	0 (0.0)	3 (5.0)	
	Severe	4 (3.3)	1 (1.7)	3 (5.0)	

<sup>a</sup>Independent sample t test; <sup>b</sup>Mann Whitney U test; <sup>c</sup>Pearson chi-squared test; <sup>d</sup>Fisher Exact test

**Table IV: Relationship between severity of lesions at challenging-to-treat areas with quality of life, mental health and itch**

		DLQI	DASS-21 TOTAL	DASS-21 DEPRESSION	DASS-21 ANXIETY	DASS-21 STRESS
<i>Severity of lesions at challenging-to-treat areas</i>						
Scalp	Correlation Coefficient	0.487	0.302	0.268	0.144	0.362
	p value	<0.001	0.001	0.003	0.116	<0.001
Face	Correlation Coefficient	0.344	0.129	0.121	0.032	0.184
	p value	<0.001	0.160	0.189	0.732	0.044
Palms	Correlation Coefficient	0.253	0.070	0.122	-0.041	0.096
	p value	0.005	0.449	0.184	0.657	0.295
Soles	Correlation Coefficient	0.250	0.073	0.168	-0.014	0.063
	p value	0.006	0.429	0.068	0.881	0.498
Genitalia	Correlation Coefficient	0.341	0.254	0.240	0.081	0.320
	p value	<0.001	0.005	0.008	0.377	<0.001
NAPSI	Correlation Coefficient	0.367	0.253	0.249	0.162	0.256
	p value	<0.001	0.005	0.006	0.077	0.005
<i>Itch severity</i>						
Itch NRS	Correlation Coefficient	0.547	0.366	0.313	0.223	0.376
	p value	<0.001	<0.001	<0.001	0.014	<0.001

Spearman correlation

**Table V: Association between the severity of challenging-to-treat areas with quality of life**

	DLQI category, n (%)					p value
	No effect	Small effect	Moderate effect	Very large effect	Extremely large effect	
Scalp						
Clear	24 (85.7)	23 (92.0)	15 (53.6)	11 (39.3)	4 (36.4)	<0.001
Almost clear	4 (14.3)	2 (8.0)	11 (39.3)	5 (17.9)	6 (54.5)	
Mild	0 (0.0)	0 (0.0)	2 (7.1)	11 (39.3)	1 (9.1)	
Moderate	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.6)	0 (0.0)	
Severe	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Face*						
Clear	25 (89.3)	23 (92.0)	18 (64.3)	16 (57.1)	6 (54.5)	0.001
Almost clear	3 (10.7)	2 (8.0)	9 (32.1)	6 (21.4)	5 (45.5)	
Mild	0 (0.0)	0 (0.0)	1 (3.6)	6 (21.4)	0 (0.0)	
Palm*						
Clear	28 (100.0)	25 (100.0)	26 (92.9)	23 (82.1)	10 (90.9)	0.105
Almost clear	0 (0.0)	0 (0.0)	1 (3.6)	4 (14.3)	1 (9.1)	
Mild	0 (0.0)	0 (0.0)	1 (3.6)	1 (3.6)	0 (0.0)	
Sole*						
Clear	28 (100.0)	25 (100.0)	27 (96.4)	24 (85.7)	10 (90.9)	0.082
Almost clear	0 (0.0)	0 (0.0)	0 (0.0)	3 (10.7)	1 (9.1)	
Mild	0 (0.0)	0 (0.0)	1 (3.6)	1 (3.6)	0 (0.0)	
Genitalia*						
Clear	28 (100.0)	23 (92.0)	24 (85.7)	19 (67.9)	8 (72.7)	0.008
Almost clear	0 (0.0)	2 (8.0)	4 (14.3)	6 (21.4)	3 (27.3)	
Mild	0 (0.0)	0 (0.0)	0 (0.0)	3 (10.7)	0 (0.0)	

Fisher Exact test

**DISCUSSION**

**Demographic characteristics of the patients**

In this cross-sectional study, psoriasis patients with challenging-to-treat areas were generally younger and exhibited a slight female preponderance, a trend consistent with findings from a Danish study by Egeberg et al. (1). However, in the Danish study, men had a higher prevalence of genital and nail involvement.<sup>1</sup> The younger age group in our study may contribute to a longer duration of psoriasis, increasing the potential for disease progression over time.

It is noteworthy that the population in our study comprises various Asian backgrounds, particularly the diverse ethnic groups of Sabah, including indigenous communities. In these settings, cultural stigma continues to pose a significant barrier to seeking mental health support. Mental illness is

often viewed as shame or personal weakness, particularly in traditional communities where spiritual or moral explanations are sometimes preferred over medical understanding. This stigma is especially strong within certain ethnic or rural populations, where mental health discussions are often suppressed or avoided entirely. A well-structured, multi-channel approach combining written materials and telecommunications would ensure better treatment outcome.<sup>23</sup>

The majority of the affected population were married and came from lower-income, working-class background, as classified by the Malaysian household income standards.<sup>24</sup> Patients may face limitations related to finances, work commitments, time availability for medical consultations,

and access to transportation. Low socioeconomic status is often associated with limited access to healthcare resources, which can affect treatment options and adherence, ultimately influencing disease management. Besides that, the involvement of challenging-to-treat areas brings discomfort and inconvenience, which can negatively impact the patients' physical and mental well-being, as seen in the Danish study and others.<sup>4,25,26</sup>

A notable finding in this study was the high prevalence of overweight and obesity, particularly among those with challenging-to-treat areas. This reinforces the established association between obesity and psoriasis severity. In addition, obesity itself can affect ones' self-confidence and many studies had shown its association with depression and anxiety.<sup>27</sup> Consequently, leading to higher treatment demands.<sup>27,28</sup>

Other comorbidities observed included hypertension (35.8%), dyslipidaemia (30.8%), diabetes mellitus (15.8%), and cardiovascular disease (4.2%). These findings were comparable to the study by Duffin et al.<sup>2</sup> It is important to note that these will all increase the financial burden to patients and our healthcare system.<sup>29</sup> A holistic approach that includes diet, lifestyle modifications and management of comorbidities alongside dermatological therapies should be adopted when managing psoriasis patients.

#### Clinical characteristics of the patients

The most common challenging-to-treat areas were the scalp (35.8%), followed by the face (26.7%), nails (38.3%), genitalia (15.0%), palms (6.7%), and soles (5.0%). A Danish study reported similar findings, with scalp (43%) and face (29.9%) involvement being most common, although fewer patients had palms and soles involvement in our study.<sup>1</sup> Despite ethnic differences, studies like the Danish Skin Cohort and Corona Psoriasis Registry highlight the scalp, face, and nails as the most affected areas in psoriasis patients, indicating these areas are consistently a major concern across populations.<sup>1,2</sup>

As all of our patients were receiving topical therapy and more than half were undergoing systemic therapy or phototherapy, the severity of lesions in challenging-to-treat areas were mostly mild to almost clear. Despite this, many patients still experienced significant impairment in QOL, along with depression, anxiety, and stress. The median NAPS I score for patients with challenging-to-area involvement was 17.5 (IQR: 1.5, 37.25), less than half of the total score. This suggests that while nail involvement is common, it doesn't always correlate with severe clinical symptoms, yet it can still significantly impact patients' lives, underlining the need for personalized treatment approaches.<sup>7</sup>

As the severity of challenging-to-treat areas increases, involvement of the scalp, face, nails, and genitalia has a profound impact on patients. These areas significantly compromise QOL and psychosocial well-being.<sup>1,9,30</sup> Scalp and face involvement is particularly visible and difficult to conceal, especially as the disease worsens. Nail psoriasis, is not only obvious but can be painful, thus further

exacerbating functional impairment and psychosocial distress.<sup>2,5-7</sup> Genital involvement is especially sensitive, affecting sexual health and relationships, as discomfort and embarrassment can occur during intimacy.<sup>9</sup> A study by Pettey et al. reported higher impairment physically and socially in those with palmoplantar psoriasis as they are sensitive functional areas.<sup>8</sup> The correlations between lesion severity in these areas with QOL and mental health necessitates the need for an integrated approach addressing these.<sup>1,2,25</sup>

Most patients had mild median PASI scores, likely due to ongoing treatment. However, challenging-to-treat areas like the scalp still significantly impacted QOL and mental health, as shown by Egeberg et al., which found higher DLQI and itch NRS scores in patients with these areas, even without severe BSA involvement.<sup>1</sup> A systematic review by Stewart et al. reported nail psoriasis patients experienced QOL impairment despite having lower PASI scores than those without.<sup>1,7,8</sup> Our findings align with these studies, as more QOL impairment is seen in those with challenging-to-treat areas despite most of them had mild PASI scores.

However, patients with challenging-to-treat areas had higher PASI scores than those without, suggesting these areas are linked to more severe disease.<sup>30</sup>

The mean itch NRS score was higher in patients with challenging-to-treat areas, many experienced moderate to severe itch despite treatment. As disease severity increased, so did itch intensity, along with greater impairment in QOL and mental health. Interestingly, a third of patients without challenging-to-treat areas reported no itching. Since itch affects QOL, personalized treatment addressing this is crucial.<sup>1,7,9</sup>

Patients with challenging-to-treat areas had significantly higher DASS scores, indicating greater psychological distress. This emphasizes that psoriasis impacts both physical and mental health, underscoring the need for integrated dermatological and psychological care.<sup>31</sup>

#### LIMITATIONS

This study has limitations like, details on treatment given, also potential recall bias on scoring of DASS-21 and hence underreporting of sensitive areas such as genital area involvement when not thoroughly examined due to patient's refusal. Future prospective study on the impact of treatment modalities on the quality of life and mental health of psoriasis patients with challenging-to-treat areas will be useful in improving patients' care.

#### CONCLUSION

In summary, this study highlights the multifaceted nature of clinical presentation of psoriasis, its associated comorbidities and its impact on QOL and mental health. The significant differences in DLQI and DASS-21 scores between patients with and without challenging-to-treat areas underscore the need for holistic and personalized treatment approaches that encompass both dermatological and psychological care.

Therefore, it is critical for clinicians to address the challenging-to-treat areas along with overall disease severity, QOL and mental health of the patients. Future research should explore these relationships to better inform clinical practice and improve patients' outcomes.

### ETHICAL CONSIDERATIONS

Approval from the Medical Research and Ethics Committee (MREC) of the Ministry of Health (MOH) Malaysia was obtained prior to initiation of the study (NMRR ID-24-01764-IDK). The study was conducted in compliance with ethical principles outlined in the Declaration of Helsinki and Malaysian Good Clinical Practice guidelines.

### CONSENT TO PARTICIPATE

Informed consent was obtained from all patients before participation in the study.

### CONSENT FOR PUBLICATION

Informed consent was obtained from all patients before participation in the study.

### DECLARATION OF CONFLICTING INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### FUNDING STATEMENT

No funding was received for conducting this study.

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# Assessment of mutagenic effects: Combined impact of nitrogen-fixing compounds and phenol on plant and animal organisms

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## ABSTRACT

**Introduction:** Rapid industrialisation, urbanisation, and increased use of agricultural chemicals have significantly contributed to environmental pollution, particularly affecting air, water, and soil quality. These pollutants, including nitrogen-containing compounds and phenol, pose mutagenic risks, potentially leading to genetic alterations and health issues in exposed populations. This study aims to evaluate the mutagenic potential of sodium nitrate, sodium nitrite, phenol, and their combinations on plant and animal cells.

**Materials and Methods:** The study utilised two experimental models: *Triticum aestivum* (soft wheat) and albino mice. Wheat seeds were treated with ten different solutions, including sodium nitrate (0.5%), sodium nitrite (0.5%), phenol (0.1% and 0.01%), and their combinations. Chromosomal aberrations in the wheat root tips were assessed using the acetocarmine staining method. For the animal model, albino mice were divided into control and experimental groups, receiving varying concentrations of sodium nitrate and phenol, both individually and in combination. Bone marrow smears were analysed for chromosomal aberrations, including fragments and rings, using metaphase plates.

**Results:** In wheat, the combined exposure to sodium nitrate (0.5%), sodium nitrite (0.5%), and phenol (0.1%) caused a significant increase in genetic alterations compared to individual treatments, with a mutation frequency 4.5 times higher than the control. In albino mice, combined exposure to high doses of phenol and nitrates induced cytogenetic changes, with the mutation frequency reaching 12.7%—1.5 times higher than the control group. Individual exposures to phenol and nitrates did not produce statistically significant mutations compared to controls.

**Conclusion:** The combination of phenol, sodium nitrate, and sodium nitrite had a synergistic mutagenic effect in both plants and animals, leading to more significant genetic damage than individual exposures. These findings highlight the need for careful management of environmental pollutants, as their combined impact may pose serious risks to ecological and human health. Further clinical studies are necessary to assess these effects in human populations.

## KEYWORDS:

*Environmental situation, atmospheric air, xenobiotics, human health, mutation, chromosome, gene*

## INTRODUCTION

In recent decades, rapid industrialisation, urbanisation, and the extensive use of agricultural chemicals have severely impacted the natural environment, contributing to the pollution of water, air, and food products. These environmental contaminants are known to have harmful effects on human health, particularly in vulnerable populations such as pregnant women and children.<sup>1</sup> Studies have shown that environmental pollution is strongly associated with various health issues, including respiratory diseases, developmental disorders, and genetic mutations.<sup>2,3</sup>

Children, in particular, are highly susceptible to environmental pollutants due to their developing bodies and faster metabolism, which can enhance the toxic effects of these substances.<sup>14</sup> Pregnant women exposed to high levels of pollutants are at increased risk of complications such as spontaneous abortions, stillbirths, and foetal developmental abnormalities, including genetic disorders.<sup>5</sup> Moreover, pollutants that cross the placental barrier can disrupt normal foetal development, leading to both immediate and long-term health consequences for the child.<sup>6</sup>

Among the many environmental pollutants, nitrogen-containing compounds (such as nitrates and nitrites) and phenol have been identified as particularly harmful.<sup>7,8</sup> Nitrogen oxides, even in low concentrations, have been shown to induce mutagenic effects, contributing to chromosomal aberrations and other genetic alterations.<sup>9</sup> Phenol, a common industrial by-product, is also recognised for its cytotoxic and genotoxic properties, especially when combined with nitrogen compounds.<sup>10</sup> Exposure to these chemicals has been linked to increased incidences of cancer, reproductive issues, and other genetic diseases in both human and animal models.<sup>11,12</sup>

Despite growing evidence of the harmful effects of nitrogen-containing compounds and phenol, there is limited research on the mutagenic effects of these substances when combined. The synergistic effects of these pollutants may exacerbate their individual toxicity, posing even greater risks to human

This article was accepted: 11 July 2025

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health.<sup>13-15</sup> Understanding the genetic impacts of such combined exposure is essential for assessing environmental risks and implementing protective measures.

Therefore, this study aims to evaluate the mutagenic effects of nitrogen-containing compounds and phenol, both individually and in combination, using laboratory models. By analysing the chromosomal aberrations in plant and animal cells, this research will provide valuable insights into the genetic risks posed by these environmental toxins, informing future strategies for reducing their harmful impacts on human health.

## MATERIALS AND METHODS

This study investigates the mutagenic potential of nitrogen-containing compounds and phenol, using both plant and animal test systems. Wheat cells were selected as a plant-based model due to their well-established use in environmental mutagenicity assessments, particularly in evaluating soil and water pollutants. Animal cells (albino mice) were chosen to provide a complementary model for assessing potential mutagenic risks in mammals, allowing the study to capture both environmental and physiological impacts. Combining these two experimental models provides a more comprehensive understanding of mutagenic risks across different biological systems, enhancing the robustness of the results.

### Plant-Based Experiment: Wheat (*Triticum aestivum*)

**Objective:** To assess the mutagenic effects of sodium nitrate ( $\text{NaNO}_3$ ), sodium nitrite ( $\text{NaNO}_2$ ), phenol, and their combinations on soft wheat (*Triticum aestivum*) through chromosomal aberration analysis.

The experiment followed the A.K. Ergashev's protocol, developed by OECD Guidelines for the Testing of Chemicals (2016)<sup>16</sup>, which is widely used for assessing chemical-induced chromosomal aberrations in plants. The ten chemical solutions used in this experiment were as follows:

#### Chemical Solutions:

1. Solution 1 : Control (pure water).
2. Solution 2 : Sodium nitrate (0.5% solution).
3. Solution 3 : Sodium nitrite (0.5% solution).
4. Solution 4 : Sodium nitrate + sodium nitrite (1:1, 0.5% solution).
5. Solution 5 : Phenol (0.1% solution).
6. Solution 6 : Phenol (0.01% solution).
7. Solution 7 : Phenol + sodium nitrate (0.1%, 0.5%, 1:1 ratio).
8. Solution 8 : Phenol + sodium nitrate (0.01%, 0.5%, 1:1 ratio).
9. Solution 9 : Phenol + sodium nitrate + sodium nitrite (0.1%, 0.5%, 0.5%, 1:1:1 ratio).
10. Solution 10: Phenol + sodium nitrate + sodium nitrite (0.01%, 0.5%, 0.5%, 1:1:1 ratio).

**Seed Treatment:** Twenty wheat seeds per group were frozen for 4 hours, then dried on filter paper and in a desiccator with potassium hydroxide for 4 days. After drying, the seeds were soaked in water for 48 hours to recover.

**Microscopic Preparation:** Root tips (5-10 mm) were fixed in acetic alcohol (3 parts ethanol, 1 part acetic acid) and stored in a refrigerator. Smears were prepared by staining root tips with acetocarmine and hydrochloric acid, followed by microscopic analysis to detect chromosomal aberrations.

### Animal-Based Experiment: Albino White Mice

**Objective:** To assess chromosomal aberrations in the bone marrow cells of albino white mice (*Mus musculus*), exposed to sodium nitrate, phenol, and their combinations at various concentrations.

**Type of Mice Used:** Albino white mice (*Mus musculus*), aged 6–8 weeks, weighing approximately 20 grams, were selected for this study. The use of this specific strain is common in mutagenicity research due to their well-documented sensitivity to environmental toxins and ease of genetic monitoring.

The study followed the Guide for the Care and Use of Laboratory Animals (8 ed.) by The National Research Council (NRC) of the United States (2011)<sup>17</sup>. The administered dosages were derived from the maximum permissible concentration (MPC) for humans and adjusted according to the body weight of the mice. These dosages were:

- Sodium Nitrate: (MPC: 2.6 mg, Half MPC: 1.3 mg, 10x MPC: 26 mg).
- Phenol: (MPC: 0.06 mg, Half MPC: 0.03 mg, 10x MPC: 0.6 mg).

#### Experimental Groups:

Group I: Administered half of the permissible concentration.

- Group I-A: Control group (no chemical exposure).
- Group I-B: Exposed to phenol solution.
- Group I-C: Exposed to nitrate solution.
- Group I-D: Exposed to a combination of phenol, nitrate, and nitrite.

Group II: Administered dosages exceeding the permissible concentration by tenfold, with the same subgroup structure as Group I.

Each group included a minimum of 15 mice. Chemical solutions were administered via oral gavage.

**Microscopic Examination:** Bone marrow smears were prepared, and chromosomal aberrations were analysed in 2,600 metaphase plates. Chromosomal abnormalities such as dicentric chromosomes, ring chromosomes, and fragments were recorded.

**Statistical Analysis:** To control for potential confounders and ensure the robustness of the findings, we employed a multivariate analysis approach. The data were analysed using relative and mean values, and correlation analysis was performed to assess the strength of the relationship between chemical exposure and chromosomal damage. Additionally, subgroup analysis was conducted to evaluate potential variability within the different experimental groups.

A dose-response analysis was performed to evaluate how the severity of genetic alterations varied with different exposure

Table I: Results of Studying the Impact of Xenobiotics on Plants

Experimental Variations	Seed Germination %	Number of Anaphase Cells	Total Genetic Alterations Count				Differences Among Solutions in Terms of Genetic Variability		p-value
			Abs.	M±m%	N1	N2	N3	N5	
Solution N1	100	166	3	1,8±1,0	X	-	-	-	p>0,05
Solution N2	100	179	17	9,5±2,2	3,2	X	-	-	p>0,05
Solution N3	95	180	23	12,8±2,5	4,1	-	X	-	p>0,05
Solution N4	95	153	21	13,7±2,8	4,0	-	-	-	p>0,05
Solution N5	60	86	3	3,5±1,9	0,8	-	-	X	p>0,05
Solution N6	70	178	18	10,1±2,3	3,4	-	-	-	p>0,05
Solution N7	95	170	25	14,7±2,7	4,4	1,7	1,5	-	p>0,05
Solution N8	80	142	24	16,9±3,1	4,6	1,0	1,7	-	p>0,05
Solution N9	95	172	34	19,6±3,0	5,8	2,7	2,0	4,5	p>0,05
Solution N10	100	231	33	14,3±2,3	5,0	1,5	0,4	1,3	p>0,05

levels. This allowed us to determine whether increasing the dosage of the chemicals resulted in a proportional increase in chromosomal aberrations. The analysis helped establish the correlation between dose and mutagenic effect, contributing to the assessment of safe exposure levels.

**Sensitivity Analysis:** Sensitivity analysis was conducted by adjusting key variables in the statistical model to ensure the results were robust across a range of assumptions.

#### Randomisation and Blinding

Randomisation was not used due to the controlled laboratory conditions and standardized exposure protocols. Blinding was not applicable as outcome assessment was based on objective cytogenetic criteria.

#### Ethical Statement for Animal Experiments

All animal procedures were carried out in accordance with the ethical standards approved by the Animal Ethics Committee of Ministry of Health of the Republic of Uzbekistan and Committee for Veterinary and Livestock Development of the Republic of Uzbekistan, following the Declaration of Helsinki and relevant national legislation. The protocol was reviewed and approved under protocol number 7/25-1953, issued by the Special Ethical Committee of MOH (SEC).

#### RESULTS

Mutagenic alterations in anaphase cells in the prepared smears were observed and analysed under the microscope. The research results concerning xenobiotic levels in higher plants demonstrated that upon exposure to pure water (N1), a 0.5% solution of sodium nitrate (N2), and a solution of 0.1% phenol with 0.5% sodium nitrate and 0.5% sodium nitrite (N9), 100% of the seeds from the solutions germinated. However, seeds exposed to solutions N5 (60%), N6 (70%), and N8 (80%) displayed the lowest percentage of growth. In the remaining four solutions, seed growth reached 95%.

From the germinated root tips of the plants (wheat), 85 smears were prepared for microscopic examination, totalling 1,656 anaphase cells analysed. Throughout the experiments, isolated and paired chromosome fragments, chromosomes, and chromatic bridges were detected. The absence of micronuclei in chromosomes and similar cellular anomalies were grouped with other types of mutations. The experiments demonstrated that harmful chemical compounds in the

external environment exhibit mutagenic activity on plant organisms.

The frequency of spontaneous mutations in the control group experiments was 1.8%. Under the influence of 0.5% nitrogen-fixing compounds, the mutation frequency increased by 5.7 to 7 times compared to the control group. The comparison group differed from the experimental observations, although the magnitude of genetic changes was not statistically significant. The germination of plant seeds decreased by 0.1% with individual exposure to a 0.1% phenol concentration. In our opinion, this phenomenon is likely associated with the death of plant cells. Reducing the impact of phenol by tenfold (to 0.01%) increased the number of genetically modified cells by five times compared to the control group.

With the combined influence of nitrogen-fixing compounds (at a concentration of 0.5%) and a 0.01% phenol solution, the frequency of mutation occurrences significantly increased compared to the control group. The results obtained from individual exposure to xenobiotics did not lead to statistically significant changes in all cases (Table I).

It is noteworthy that the combined exposure of 0.1% phenol with high concentrations of sodium nitrate and sodium nitrite (0.5% solutions) led to more profound genetic alterations not only in the control group but also in their separate exposures. Such alterations were observed to be 2.7 times greater than the impact of nitrates alone, 2 times greater than the effect of nitrites alone, and 4.5 times greater than the influence of 0.1% phenol. The outcomes of these experiments provide the opportunity to identify combinations of xenobiotics that yield more potent and effective effects amidst the various combinations tested.

The experiments following Method 2 commenced with the administration of high doses of substances to the experimental animals (more than ten times the MPC).

As shown in Table II, the frequency of spontaneous mutations in the comparison group was relatively low, at  $8.7 \pm 1.3\%$ , as substantiated by the analysis of metaphase plates.

The effects of the studied chemical substances at doses exceeding the MPC by tenfold vary depending on their application. No statistically significant changes in mutational processes were observed upon separate exposure

Table II: Results of Investigating the Impact of High Doses of Xenobiotics on Animals

Experimental variations	Number of metaphase plates	Spectrum of aberrations					Cytogenetic changes		Differences among experiments in terms of genetic variability			p-value
		Isolated fragments	Paired fragments	Dicentric chromosome	Acentric chromosome	Centric ring	Abs.	M±m%	N1	N2	N3	
N1 - control animals	496	22	7	10	2	2	43	8,7±1,3	X	-	-	-
N2 - experiment-1 phenol	329	15	2	10	2	0	29	8,8±2,0	-	X	-	p>0,05
N3 - experiment-2 nitrate	163	7	1	5	2	0	15	9,2±2,3	-	-	X	p>0,05
N4 - experiment-3 phenol-nitrate	831	35	25	36	8	2	106	12,7±1,2	2,4	2,0	1,3	p<0,01

Table III: Results of Investigating the Impact of Low Doses of Xenobiotics on Animals

Experimental variations	Number of metaphase plates	Spectrum of aberrations					Cytogenetic changes		Differences among experiments in terms of genetic variability			p-value
		Isolated fragments	Paired fragments	Dicentric chromosome	Acentric chromosome	Centric ring	Abs.	M±m%	N1	N2	N3	
N1 - control animals	496	22	7	10	2	2	43	8,7±1,3	X	-	-	-
N2 - experiment-1 phenol	57	2	1	2	1	0	6	9,0±3,4	-	X	-	p>0,05
N3 - experiment-2 nitrate	441	17	11	23	2	8	61	13,8±1,6	-	-	X	p<0,01
N4 - experiment-3 phenol-nitrate	275	12	12	14	0	5	43	15,6±2,2	2,8	2,1	0,6	p<0,01

to nitrates and phenol ( $8.8 \pm 2.0\%$  and  $9.2 \pm 2.3\%$ , respectively, with  $p > 0.05$ ). However, with the combined action of nitrates and phenol at the same doses, genetic alterations significantly intensified compared to the control group, reaching  $12.7 \pm 1.2\%$  (1.5 times higher). Changes resulting from separate exposure to nitrites and nitrates did not significantly differ statistically from each other.

The obtained results provide grounds to conclude that high doses of phenol and nitrates exhibit cytotoxic effects and suppress genetic alterations. Meanwhile, it is confirmed that a stronger manifestation of genetic changes in plants occurs with the combined influence of chemical agents.

Upon exposure to the preparations under study at concentrations half of the MPC, the following results were observed (Table III).

As evident from Table III, the number of mutated cells and identified mutations upon exposure to phenol at 0.5 MPC (individually) amounted to  $9.0 \pm 3.4\%$ . This result is not statistically significant when compared to the control group ( $p > 0.05$ ). The total number of genetic alterations when exposed to a sodium nitrate solution concentration two times less than the MPC was  $13.8 \pm 1.6\%$ , which is 1.6 times higher than the control group ( $p < 0.01$ ). With the combined influence of sodium nitrate and phenol, the level of genetic alterations increased by 1.8 times compared to the control group, reaching  $15.6 \pm 2.2\%$  ( $p < 0.01$ ).

## DISCUSSION AND CONCLUSION

The results of our study highlight the mutagenic potential of phenols and nitrates, as evidenced by the chromosomal aberrations observed in both wheat and albino mice models.<sup>18,19</sup> However, it is crucial to carefully consider how these findings may relate to human health. While the mutagenic effects identified in plant and animal models suggest a potential risk for humans, direct extrapolation must be approached with caution. Biological responses to chemical exposures can vary significantly between species due to differences in metabolism, physiology, and genetic makeup.<sup>20</sup> Therefore, further clinical studies are essential to accurately assess the risks these compounds pose to human health, particularly with regard to potential carcinogenic effects and genetic damage.

Additionally, potential sources of bias, such as environmental variability and interspecies differences, must be acknowledged. Factors such as variations in environmental conditions, genetic diversity among test subjects, and differences in exposure levels can influence the outcomes of mutagenicity studies.<sup>21</sup> These variables may affect the robustness and generalisability of our findings, underscoring the need for a comprehensive assessment that includes a range of environmental conditions and a broader selection of biological models.

Moreover, while our study focused on chromosomal aberrations, it is important to delve deeper into the underlying mechanisms driving these genetic changes. Understanding processes such as DNA repair pathways,

specific gene mutations, and the molecular interactions of phenols and nitrates with genetic material is crucial for elucidating how these compounds exert their mutagenic effects. Insights into these mechanisms can enhance our understanding of the biological impact of chemical exposures and help inform strategies for mitigating their potential risks.

In conclusion, while our findings contribute valuable data regarding the mutagenic potential of phenols and nitrates, further research is necessary to explore their implications for human health, address potential biases, and investigate the molecular mechanisms underlying the observed genetic alterations. A comprehensive approach will provide a clearer understanding of the risks associated with these compounds and inform public health guidelines.

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# Poor waste disposal and effect on health: An insight and review

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## ABSTRACT

**Introduction:** The generation of waste products, including urine, faeces, and household items such as glass and plastic, has increased in line with the growth of the world's population. Physical, psychological, and social issues can invariably arise if these wastes are not properly managed, which has a direct effect on the population's health and is the source of the nation's productivity. Waste dumping has been a persistent problem, despite the government's efforts to educate consumers on proper waste dumping. Despite regular enforcement activities, this socially unacceptable habit persists.

**Methodology:** Various studies on waste disposal and its potential deleterious effects on the health of humans, animals, and the environment were reviewed and included in this narrative review to summarise the nature of the problem and its health effects. These include both local and international articles on this subject. This is to ensure that various strategies can be developed to manage this important and ever-persisting issue.

**Results and conclusion:** The literature reviewed have been able detailed the physical, psychological, and social issues of improper waste disposal that may affect the health of the population. Taking care of the environment is a top priority, as are modern issues of climate change and its effects on the nation's coffers. Enhancing public education, promoting community-based environmental activities such as gotong-rojong, using modern technology or methods, and stricter enforcement will ensure that waste is disposed properly. In turn, this will ensure the sustainability of Mother Earth for future generations.

## KEYWORDS:

*Waste dumping, health, physical, psychological, social*

## INTRODUCTION

Malaysia is one of the fastest developing countries in the world, with the ambitious aim of achieving a developed nation status in the next 30 years. Along with the growth in urbanization, there is also a residual problem of waste generation. The peninsular Malaysia population alone produced about 19,100 tons per day of waste in 2005 or an average of 0.8 kg per capita per day, up 20% from the amount in 2001.<sup>1</sup> This figure is unfortunately expected to

grow further as Malaysia is already a one-trillion economy with an equally thriving birth rate. The latest data from 2024 show that Malaysia generates approximately 40 thousand tons of municipal solid waste daily, approximately 1.2 kg per person.<sup>2</sup> Food waste alone equates to 260 kg per person. This increase has been catalysed by urbanization, changing eating habits and population growth.<sup>2</sup> As reported in USA market intelligence website, Malaysia is seriously facing constraints in having sufficient space for waste disposal, further risking possible consequences in terms of health and environmental issues.<sup>2</sup>

Landfilling is the primary source of waste disposal in Malaysia.<sup>3</sup> Solid waste is mainly generated from the municipal council (64%), followed by industrial waste at 25%, and commercial and construction waste that makes up 8% and 3% of the total waste, respectively.<sup>4</sup> In landfills, recyclable materials can make up 80% of the total solid waste composition.<sup>5-6</sup> The problem is not waste generation per se, but improper waste management resulting from waste dumping in many places other than homes, that is, on the roadside, into water sources such as rivers and beaches and even public places such as the parks.

This can in turn lead to many ill effects on health, namely physical, psychological, and social effects that will be explored in this article. Malaysia needs to step up its actions to regulate the unsanitary activity of improper waste dumping. After all, a healthy population is needed to drive the economy of the country, especially in the productive and young groups, which often become victims of tropical and vector-borne diseases, such as typhoid, cholera, malaria, and dengue fever. Therefore, this narrative review aims to showcase poor waste disposal and its effect on health worldwide, specifically in Malaysia. This eventually can help to draw up effective strategies to manage waste disposal, thereby reducing its potential health effects on the inhabitants of the earth.

## Magnitude of the Problem

The magnitude of this potential health risk is enormous. Worldwide, billions of tons of waste are generated annually. Worse still is the fact that most of this waste is not recyclable and goes back into the vulnerable soils or water sources that will face the ultimate brunt from the uncivilized actions of waste dumping, despite the provision of adequate facilities for proper rubbish disposal in most countries.

*This article was accepted: 30 May 2025*

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This is a problem of worldwide magnitude, affecting all five continents, from developed to the least developed countries. While improper waste management leads to the propagation of modern diseases such as cancers in the northern hemisphere (the so-called developed countries), in the southern hemisphere, which holds some of the poorest nations on earth, water-borne diseases such as cholera, typhoid, and malaria predominate, resulting in millions of fatalities per year, which are equal to some developed countries with smaller population, such as New Zealand and Iceland.

Comparing municipal waste generation by states in Malaysia, out of the 9,820 tonnes of waste generated, the central region states of Kuala Lumpur and Selangor contributed to one-third of waste generation, which is more than their population ratio in Malaysia (28.4%).<sup>7-8</sup> In comparison, the east coast states of Pahang, Kelantan, and Terengganu only generated 517 tons of waste as compared to their population ratio of 14.1%.<sup>7-8</sup> This indicates that the central region states are at greater risk of inappropriate waste disposal due to rapidly reducing landfill areas, relatively lower recycling habits, lack of infrastructure, and skills in waste management.<sup>2</sup>

#### Health effects

There are various negative effects on physical and psychological health, along with concomitant possible negative social outcomes that will be summarised here.

##### i) Physical health effects

Unscrupulous waste dumping can have many adverse effects on physical health. Poor waste management can lead to various physical illnesses. This can be divided into gastrointestinal infections such as typhoid, cholera, worm infestation, hepatitis A, and food poisoning; serious blood infections such as dengue fever and leptospirosis due to the presence of mosquito breeding grounds and flies and rodents; and respiratory illnesses such as asthma and irritation of the skin, nose, and eyes due to exposure to landfill gases such as methane, carbon dioxide, sulfur dioxide, and nitrogen dioxide.<sup>9-18</sup>

Improper sharp disposal can lead to accidental needle-stick injuries that may lead to serious diseases such as HIV/AIDS and, hepatitis B and C. In terms of water pollution, the size of total waste in the Pacific Ocean is already twice the mammoth size of the United States of America, thereby serving as a suitable environment for the growth of these undesirable bacterial and protozoal infections.<sup>19</sup> This may lead to various manifestation of body and skin irritation due to the use of the polluted water, as well as infecting sea lives that are later consumed as meals by unsuspected consumers.<sup>20</sup>

Heavy metals also present a clear danger to human health if it is improperly disposed. This includes metals such as cadmium, lead, copper, aluminium, and zinc.<sup>21</sup> Cadmium may lead to serious problems like chronic kidney disease, cancer and teratogenic effect on the fetus. Lead, a common metal found in paints, can lead to behavioral problems and lower IQ, especially in children.

Aluminum can lead to serious damage to the central nervous system and may worsen the prognosis of patients with chronic kidney disease. Similarly, Copper causes serious damage to the brain. Zinc is more toxic to fish than humans, but may become toxic if the affected fish are eaten by human.<sup>21-22</sup>

The discovery of microplastics in areas of waste disposal is also another great worry.<sup>23</sup> Various complications due to microplastic consumption have been reported in aquatic and land animals, such as reduced fertility, organ failure, and even death.<sup>24-25</sup> These serious effects may also theoretically extend to humans if contaminated meat is consumed in large amounts.<sup>23</sup>

##### ii) Psychological health effects

Besides its effects on physical health, there are also multiple psychological effects secondary to improper waste disposal. Studies conducted in least developed countries such as Peru and Brazil have shown that having diarrheal disease in the first two years of life may affect cognitive functioning in children when the child enters school.<sup>26</sup> Yet other studies have demonstrated that malnutrition and stunting secondary to chronic intestinal worm infestation resulting from unsanitary water resources, may affect the child's future psychosocial development.<sup>27</sup>

There is also evidence of an increased risk of inattention and anxiety in stunted children.<sup>26</sup> Adults are also more prone to central nervous system toxicity due to heavy metals, as described above, and serious mutagenic risk that may eventually lead to cancer.

Other psychosocial effects such as disturbing odor, unsightly waste, and cognitive and stress-related problems remain a true and present danger of persistent improper waste disposal.<sup>9</sup> This is especially true for odors from landfill sites, and their physical appearance, which can affect the income of nearby residents by undermining their livelihoods and lowering their residential worth or tourism potential, affecting the tranquillity and quality of life that often comes with increase in stress and anxiety level.<sup>28-29</sup>

##### iii) Social effects

Improper waste disposal can also have deleterious social implications. Studies have shown that places with inadequate sanitation have a high propensity for violence, social inhesions and insecurity.<sup>30</sup> This is can even be seen in certain low-cost residences in Malaysia where the incidence of vandalism and crime is somewhat high. Other possible social effects include poor social interactions and propagation of drug use.<sup>30</sup>

Growing up in an environment surrounded by poor sanitation and waste disposal can also lead to the propagation of the above social problem.<sup>31</sup> This is a serious problem that can seriously affect national aspiration to become a developed nation.

A case study conducted in Kuala Langat and Sepang, Selangor has shown that proper waste management policies are needed to prevent improper waste disposal by waste

dealers that can further contribute to environmental degradation.<sup>8</sup> Another case study on household waste management in Malaysia also noted that a high volume of organic waste is dismantled in landfills and produces a toxic poisonous substance called leachate and greenhouse gases such as nitric oxide and methane, which are 21 times more potent than carbon dioxide.<sup>32-33</sup>

In addition, depletion of natural resources and the resultant climate changes with extreme hot weather and severe flooding continue to plague the nation, partially contributed by issues in waste disposal.<sup>34</sup> In addition, open dumping may also lead to air pollution through waste burning and offensive odors along with surface and soil contamination by leachate.<sup>34</sup>

### CONCLUSION AND RECOMMENDATIONS

Improper waste disposal is a real problem that must be addressed urgently and systemically. Failure to tackle this problem will lead to a vicious cycle of waste disposal, resulting in poor health, which in turn leads to improper waste disposal due to strain on income used to treating the resultant ill health.

The civic-mindedness of the community needs to be addressed to ensure that this problem does not get out of hand. Simple measures such as regular gotong royong together with proper waste disposal practices, coupled with enforcement by the authorities, will go a long way together, ensuring the sustainability of Mother Earth and life. Sustainable landfilling may be a viable solution for proper disposal of waste products.<sup>35-36</sup>

Studies in Malaysia have shown that community participation in environmental activities had a positive influence on healthy environment-related knowledge.<sup>37</sup> This in turn correlates positively with positive environment-related behaviors, participation, and attitudes.<sup>38</sup> This reiterates the old age statement that a behavior that shows responsibility to the environment will in turn relate to consumption activities that will benefit and cause less harm to Mother Earth, more than other substitutable activities per se.<sup>38-39</sup>

Many initiatives have been launched by the Malaysian government to address ever-growing solid waste disposal issues. This includes enforcing the Extended Producer Responsibility (EPR) policy, which makes it mandatory for importers and manufacturers to be in charge of the waste disposal of their products and not only of its' sales.<sup>3</sup> This is further enhanced by reinforcing the 2007 Solid Waste and Public Cleansing Management Act, which clearly spells out stiff penalties for violations and emphasizes holistic waste management practices. Another program being conducted is the National Strategic Plan for Solid Waste Management 2019–2030, aimed at lowering waste generation and its' management and encouraging recycling practices.<sup>3</sup>

Of course, great efforts to organizing public education and awareness campaigns on waste management and segregation, as mentioned above, including the 3R (reduce,

reuse, recycle) concept, also help in saving the environment.<sup>3,40</sup> These efforts are expected to bear positive outcomes in the years to come.

In recent years, the use of advanced technologies or methods for waste disposal has helped further sustainable waste management. This includes mechanical methods such as biological pre-treatment, composting, and refuse-derived fuel production.<sup>41-42</sup> It is also possible to separate the source of dry waste at home to ease the overall disposal services.<sup>43</sup>

In summary, enhancing public education, promoting community-based environmental activities such as gotong royong, using advanced technology or methods, and stricter enforcement will ensure that wastes is disposed properly. In turn, this will ensure the sustainability of Mother Earth for future generations.

### Declarations

Ethical approval and consent to participate: No ethical approval was required for this review as it is based on published research and does not involve any new studies of human participants or animals performed by any of the authors.

Availability of data and material: Data sharing is not applicable to this article because no datasets were generated during the current study.

Declaration of Conflicting Interests: The authors declare no competing or conflict of interest concerning this article's research, authorship, and publication.

Funding and ethical approval: The authors wish to convey that this study was conducted without the aid of any financial support. No grants or funds were received from public, commercial, or nonprofit funding entities to facilitate this study.

Authors' contributions: All authors have reviewed the manuscript, believe that it represents valid work, and approve it for publication. All the authors participated in the research design, research performance, data analysis, and manuscript writing.

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# A systematic review of water-soluble contrast use in videofluoroscopic examination of dysphagia

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## ABSTRACT

**Introduction:** The Videofluoroscopic Swallow Study (VFSS) is a key diagnostic tool for evaluating swallowing function, providing dynamic visualization of bolus transit and swallowing mechanism. Traditionally, barium is used as the contrast medium due to its effectiveness in highlighting anatomical and functional aspects of swallowing. However, water-soluble contrast (WSC) agents, which differ in osmolarity, viscosity, and iodine atom-particle ratio, have emerged as alternatives and their optimal use in VFSS remains unclear. This systematic review investigates the utilization of WSC agents in VFSS.

**Materials and Methods:** This systematic review was conducted following the PRISMA 2020 guidelines, extensively analyzing WSC agents use in VFSS. Searches were conducted across PubMed, Scopus, and Google Scholar using keywords “Water-Soluble Contrast, Videofluoroscopic, High-Osmolar Water-Soluble Contrast, and/or Low-Osmolar Water-Soluble Contrast”. A total of 574 papers were initially identified.

**Results:** Nine studies (2013–2021) investigated the use of WSC agents in VFSS. The studies varied in methodology and WSC agents used, with no standardized protocols identified. Findings indicated that low-osmolar WSCs may reduce the risk of complications, such as aspiration and pulmonary edema, compared to barium sulfate. WSC agents were utilized in high-risk populations, such as those with head and neck surgeries or neurological conditions.

**Conclusion:** WSC agents may serve as a viable alternative to barium sulfate in VFSS, particularly for high-risk patients. However, further research is needed to compare their diagnostic accuracy, patient outcomes, and safety profiles, as well as to establish standardized protocols for their use in dysphagia evaluation.

## KEYWORDS:

*Videofluoroscopic Swallow Study (VFSS), water-soluble contrast (WSC) agents, dysphagia, osmolarity, contrast media, systematic review*

## INTRODUCTION

Dysphagia evaluation is a critical component of clinical practice for speech-language pathologists (SLPs), and it

involves both instrumental and non-instrumental assessments to diagnose and manage swallowing disorders accurately. Non-instrumental assessments, such as questionnaires, water swallow tests, and clinical bedside examinations, provide valuable preliminary insights into swallowing difficulties.<sup>1</sup> Instrumental assessments, including Fiberoptic Endoscopic Evaluation of Swallowing (FEES) and Videofluoroscopic Swallow Study (VFSS), provide a detailed and objective examination of the swallowing process.<sup>2</sup> VFSS employs radiological imaging to assess swallowing mechanics.<sup>3</sup> By using VFSS to analyze anatomical landmarks from the oral cavity to the esophagus, SLPs can accurately diagnose swallowing dysfunction and develop appropriate rehabilitation strategies.<sup>4</sup>

Central to the VFSS procedure is using contrast agents to enhance visualization of the bolus within the upper digestive tract. The gold standard contrast is Barium Sulfate, which is odorless, white, and poorly soluble in water.<sup>4</sup> Barium Sulfate is preferred for its superior mucosal coating, high contrast, and attenuation properties, which facilitate the detection of subtle leaks.<sup>5</sup> However, the use of Barium Sulfate poses significant risks, including intestinal infarction in patients with gastrointestinal perforations and increased mortality rates in individuals with esophageal fistulas, particularly in pediatric populations.<sup>6</sup> In contrast, water-soluble contrast (WSC) agents offer an alternative with varying osmolarity levels. WSCs are categorized into four types: ionic monomeric, nonionic monomeric, ionic dimeric, and nonionic dimeric.<sup>7</sup> The distinguishing features of these agents include osmolality, viscosity, and the iodine atom-to-particle ratio. Osmolality refers to the concentration of solute particles in the contrast medium, while viscosity describes the fluid's density and internal resistance to motion. For example, Gastrografin, a high-osmolar contrast, has an iodine concentration of 367 mg/mL and is administered in doses of 30-100 mL, whereas Omnipaque 350 (Iohexol) contains 350 mg/mL iodine and is given in doses of 50-150 mL.<sup>8</sup> High-osmolar contrasts like Gastrografin may cause pulmonary edema due to fluid shifts into the lungs upon aspiration.<sup>9</sup> In contrast, low-osmolar WSCs such as ioxaglate sodium have not been associated with pulmonary edema (9).

Guidelines from professional bodies, such as the Royal College of Speech and Language Therapists (RCSLT) in the United Kingdom, recommend the use of WSCs for initial testing in patients suspected of being at high risk of

This article was accepted: 12 June 2025

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aspiration.<sup>10</sup> Similarly, the Western Australia Country Health Services advocates for the use of WSCs in patients with a risk of gastrointestinal perforation.<sup>11</sup> These recommendations underscore the importance of selecting the appropriate contrast agent, particularly for high-risk populations, to enhance patient safety and diagnostic accuracy. This systematic review aims to synthesize research from the past decade on the use of high- and low-osmolar WSCs in VFSS, highlighting their potential benefits and risks in dysphagia evaluation.

## MATERIALS AND METHODS

### Search strategy

PubMed, Scopus, and Google Scholar databases were searched to retrieve articles published between 2013 and 2023. The search strategy involved using a combination of key terms and their variations. The key terms included WSCs (WSC), Video-Fluoroscopy (VFSS), High-Osmolar Water-Soluble Contrast (HOWSC), and Low-Osmolar Water-Soluble Contrast (LOWSC). This approach guaranteed a thorough exploration of the relevant literature on using WSC agents in VFSS evaluations.

### Eligibility criteria

The inclusion criteria for eligible studies included articles, books, clinical trials, randomized controlled trials, and systematic reviews. Exclusion criteria encompassed studies not published in English, those using WSCs for purposes other than VFSS, and studies that did not specifically utilize WSC agents.

### Selection process

This systematic review followed the PRISMA 2020 Flow Chart (Figure 1) to ensure a structured and transparent process for documenting study selection, including identification, screening, eligibility assessment, and inclusion.

### Data extraction and analysis

The first author independently screened the titles and abstracts according to the specified criteria. Full-text evaluations were then performed on the studies deemed eligible from this initial review. Following a comprehensive assessment of these full texts, the reviewer selected the studies for inclusion. Relevant data, including author(s), publication year, country of origin, methodology, and key socioeconomic factors, were extracted and thematically analyzed. All authors reviewed and double-checked the papers included in the final systematic review.

## RESULTS

This systematic review analyzed nine studies assessing the efficacy and utility of WSC agents in evaluating and managing dysphagia across various patient populations and clinical contexts. The characteristics of the included studies are summarized in Table I. The study designs included three retrospective studies, two prospective cohort studies, two cross-sectional descriptive studies, one literature review, and one double-masked randomized controlled trial. The research spanned from 2013 to 2021 and encompassed diverse geographical locations, including the United States, Finland, Spain, India, Hong Kong, and Korea.

### Detection of Aspiration and Leakage

Several studies have demonstrated the effectiveness of WSC agents in detecting aspiration and leakage during VFSS. Iohexol, a low-osmolar WSC, was shown to detect aspiration in 22% of examinations and was particularly effective in detecting extraluminal leakage in 58% of cases when used alone.<sup>12</sup> The study further emphasized that Iohexol had no adverse effects, making it a safer option compared to barium sulfate, especially in patients at risk of aspiration. In addition, Gastrografin, another WSC, was used to detect pharyngeal leaks post-laryngectomy.<sup>13</sup> The study found that small volumes of Gastrografin were effective in detecting leaks in 46% of cases, which helped prevent the development of pharyngocutaneous fistulas and aided in conservative management without the need for surgical intervention.

### Risk of Pulmonary Complications

The safety profile of WSC agents, particularly in pediatric and high-risk populations, was consistently highlighted across studies. In pediatric patients, Iohexol demonstrated greater sensitivity than barium in detecting aspirations without increasing the risk of pulmonary complications such as pulmonary edema or aspiration pneumonia.<sup>14</sup> Additionally, no allergic reactions or chemotoxicity were reported following WSC use, nor was there any prolongation of discharge intervals, suggesting that WSC is a safer alternative for vulnerable populations.<sup>14</sup>

### Impact on Dysphagia Management

The use of WSC in VFSS poses significant implications for dysphagia management particularly in complex clinical populations. In pediatric patients with neurological impairments, Visipaque enabled more accurate identification of swallowing difficulties, which led to the implementation of individualized treatment strategies.<sup>15</sup> The study reported that 70% of the children had dysphagia, with 58% being moderate to severe cases, underscoring the importance of accurate and safe contrast agents in the management of dysphagia in complex cases. Supporting these findings, a broader review highlighted the adaptability of WSC agents across various clinical scenarios, reinforcing their role in comprehensive dysphagia evaluation.<sup>18</sup> Furthermore, in a clinical protocol involving subacute stroke patients, respiratory muscle training was associated with reduced aspiration risk. In that setting, Visipaque was incorporated into the diagnostic process, contributing to improved management outcomes.<sup>16</sup>

### Special Considerations in Post-Surgical and Neurological Patients

In patients with specific conditions, such as post-surgical or neurological impairments, selecting the appropriate contrast agent is crucial. Risk factors for aspiration and penetration were identified in individuals with acute traumatic cervical spinal cord injuries using Omnipaque, a WSC agent, emphasizing the value of WSC for evaluating severity in patients with higher PAS scores and facilitating early intervention.<sup>17</sup> Comparisons of diagnostic tools in patients with motor neuron disease revealed that while screening questionnaires like the EAT-10 offer initial assessment, VFSS with WSC remains essential for accurate diagnosis and management.<sup>18</sup> Additionally, in patients with chronic aspiration following radiotherapy for head and neck cancer

**Table I: Summary of the articles reviewed**

Title	Study details	Research Objectives	Population	Methodology
The Use of Low-Osmolar Water-Soluble Contrast In Video-fluoroscopic Swallowing Exams	Authors: Julie A. Harris et al. Year: 2013 Country: USA Design: Prospective cross-sectional study.	1) To document usage of non-ionic water-soluble contrast, mainly iohexol and barium contrasts, in adults who underwent fluoroscopic examination. 2) To provide the clinical use indication of using both contrasts.	Adult patients undergoing fluoroscopic exams of the pharynx and esophagus Experimental Group: Iohexol: 36 patients Iohexol and Barium: 197patients Control group: Barium: 206 patients (Total: 1978 VFSS)	Four experienced gastrointestinal radiologists and 12 SLP's rated the fluoroscopic exams. VFSS analyses were based on leakage detection and leakage percentage for each type of contrast used.

**Outcomes:**

Aspiration occurred in 22% of exams. Vestibular penetration occurred in 38% of exams. Extraluminal leakage of contrast was observed in 4.6% of exams. In aspiration cases, iohexol was used alone (8%), iohexol and barium were used together (45%), and barium was used alone (47%). In extraluminal leakage cases: iohexol alone (58%), iohexol and barium (31%), and barium alone (11%). No adverse effects were observed with iohexol use. Small amounts of aspirated barium and extraluminal barium were noted.

**Review:**

Iohexol is effective and safe as a screening contrast agent. It reduces the risk of large-scale aspiration and leakage of barium. Barium remains useful but can lead to complications in some cases; thus, iohexol may be preferred to mitigate risks.

Title	Study details	Research Objectives	Population	Methodology
Risk factors for laryngeal penetration-aspiration in patients with acute traumatic cervical spinal cord injury (TCSCI).	Authors: Tiina Ihalainen et al. Year: 2017 Country: Finland Design: A prospective cohort study.	1) To inspect the risk factors of patients with acute traumatic cervical spinal cord injury for aspiration-penetration	37 adult patients with acute traumatic cervical spinal cord injury (TCSCI) (mean age: 61.2 years).	Ominpaque 350mg/ml. Three different volumes: 5ml, 10ml, 20 ml. A clinical swallowing trial and VFSS were performed within 28 days post-injury. Patients were divided into two groups based on the Rosenbek Penetration-Aspiration Scale (PAS) score (range 1-8) of each patient. Group 1- penetrator/aspirators (PAS score ≥3) and Group 2-non-penetrator/aspirators 15 (PAS score ≤2).

**Outcomes:**

VFSS results show that 51.4% of the patients scored higher than three on the aspiration penetration scale, while 48.6% scored two or less. Seventy-four percent had silent aspiration. There was a statistical difference in the need for bronchoscopy between the two groups who aspirated or penetrated liquids. In addition, the group that showed aspiration and penetration had a higher number of vertebral fractures and cervical spine fractures.

**Review:**

The identified risk factors associated with penetration-aspiration in TCSCI patients were coughing, throat clearing, choking, and changes in voice quality; those risk factors should be considered for further evaluation of suspected pharyngeal dysfunction to avoid further complications. VFSS using WSC is a valuable complement in managing swallowing issues for these patients.

Evaluation of dysphagia. Results after one year of incorporating Video-Fluoroscopy into its study	Authors: Ruth García Romero et al. Year: 2018 Country: Spain study design: cross-sectional descriptive study	1) To analyze Video-Fluoroscopy results after prescribing an individualized treatment plan for patients after one year in the center	61 children with neurological disabilities	Visipaque with three different consistencies (thin and thick liquids and pudding) and three different volumes, 3ml, 5ml, and 10 ml, was used in VFSS.  Additional variables that were measured include age, pathology, degree of neurological damage, type and severity of dysphagia (oral, pharyngeal, oesophageal), aspirations, prescribed treatments, and improvements in nutrition and respiration.
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**Table I: Summary of the articles reviewed**

**Outcomes:**

70% of the children's dysphagia was detected, and 58% of it was moderate to severe. Aspirations and/or penetrations were recorded in 59% of cases, of which 50% were silent. Nutritional and respiratory improvements were noted in patients after VFSS. Dysphagia was also effectively managed.

**Review:**

VFSS using WSC implementation led to high diagnostic accuracy and effective treatment for patients. The findings support the inclusion of VFSS in pediatric diagnostic protocols to improve patient outcomes in terms of nutrition and respiratory health.

ACR Appropriateness Criteria Dysphagia	Authors: Levy et al. Year: 2019 Country: USA study design: A literature review study	1) To extensively review the current medical literature from peer-reviewed journals and evidence-based methodologies used to create an appropriate procedure for imaging and treatment of specific cases of swallowing disorders.	Patients with symptoms of dysphagia.	The guideline was developed by a multidisciplinary team of experts using a thorough review of literature from peer-reviewed journals, the RAND/UCLA Appropriateness Method, and the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) to assess the suitability of imaging and treatment procedures for specific clinical situations.
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**Outcomes:**

An evidence-based guideline - The American College of Radiology Appropriateness Criteria for Dysphagia.

**Review:**

Modified barium evaluation is the golden standard for assessing patients with swallowing disorders. However, in some special considerations, where swallowing needs to be assisted immediately post-operation for patients who underwent head and neck surgery, Diatrizoate meglumine and diatrizoate sodium solution, or Iohexol WSC, is preferable, especially if a leak is suspected due to a fistula.

Title	Study details	Research Objectives	Population	Methodology
Tubed Supraglottic Laryngeal Closure to Treat Chronic Aspiration After Radiotherapy for Head and Neck Cancer	Authors: Peter K. M. Ku et al. Year: 2020 Country: Hong Kong study design: A retrospective study	1) To investigate the long-term effect of surgical treatment on patients who suffer from chronic aspiration due to radiotherapy on the head and neck	17 adult patients. - 15 with tubed supraglottic closure (TSLC) - 2 with total laryngectomy	Patients who had radiotherapy for head and neck cancer and subsequently required laryngectomy or TSLC for chronic aspiration pneumonia. Swallowing function assessed using the Functional Oral Intake Scale (FOIS) and the Swallowing Performance and Status Scale (SPSS). The type of WSC was unspecified.

**Outcomes:**

There was significant improvement on both swallowing scales after total laryngectomy or tubed supraglottic closure at 12, 24, and 36 months. However, the two patients with total laryngectomy continue to suffer from recurrent episodes of aspiration pneumonia after surgery.

**Review:**

The study provides evidence supporting TSLC as a preferable option over laryngectomy for managing chronic aspiration in patients with a history of radiotherapy for head and neck cancer. The type of WSC agent in VFSS was not specified.

Efficacy of small-volume gastrografin Video-fluoroscopic screening for detecting pharyngeal leaks following total laryngectomy	Authors: M Narayan et al. Year: 2020 Country: India Study design: A retrospective case-control study	1) To investigate the ability to use gastrografin contrast in detecting pharyngeal leaks after total laryngectomy	137 adult patients under 60 years of age  Experimental Group (n = 52): - 48 males and four females  Control group (n= 85): - 80 males and five females	3-5ml gastrografin water-soluble.  The control group was assessed clinically for development of pharyngocutaneous fistulae, while the experimental group underwent small-volume (3-5 ml) post-operative gastrografin VFSS.
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**Table I: Summary of the articles reviewed**

**Outcomes:**

Pharyngeal leak was detected in 46% of the study group and 4 had pharyngocutaneous fistulae development after laryngectomy compared to 28% of patients in the control group with 6 requiring surgical correction.

**Review:**

The use of small volumes of gastrografin during VFSS facilitates the detection of even minor pharyngeal leaks, thereby reducing the risk of developing a pharyngocutaneous fistula and preventing wound dehiscence.

Evaluation of Dysphagia in Motor Neuron Disease. Review of Available Diagnostic Tools and New Perspectives	Authors: Elisabet Romero Gangonells et al. Year: 2020 Country: Spain Study design: A transversal, unicentric observational study	1) To investigate the sensitivity and Specificity of the EAT-10 and SwalQoL questionnaires and the ALS-SS and FOIS scales compared to the gold standard technique, VFSS.	45 adult patients with motor neuron disease.	Nectar, thin liquid, and pudding consistencies in 5ml, 10 ml, and 20ml volumes, mixed with an unspecified type of WSC in VFSS. Results were compared to Eating Assessment Tool (EAT-10), and SwalQoL questionnaires, as well as the ALS-SS and FOIS scales.
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**Outcomes:**

Of the 45 patients, 37 patients were diagnosed with dysphagia while 8 had no dysphagia based on VFSS and Dysphagia Outcome and Severity Scale. Only 28 patients were diagnosed with dysphagia based on the ALS-SS and EAT-10 questionnaires and 12 patients based on the FOIS scale.

**Review:**

None of the evaluated questionnaires demonstrated superior sensitivity, but the SwalQoL revised FS stood out for its perfect specificity. With modifications to its symptom frequency section, the SwalQoL revised FS may be a valuable tool for clinically assessing dysphagia, particularly in accurately identifying patients without the condition. The ALS-SS exhibited the highest validity as a severity scale for dysphagia. The type of WSC agent in VFSS was not specified.

Swallowing study using water-soluble contrast agents may increase aspiration sensitivity and antedate oral feeding without respiratory and drug complications: A STROBE-compliant prospective, observational, case-control trial.	Authors: Chang Ho Hwang Year: 2020 Country: Korea Study design: prospective, observational, case-control cohort trial	1) The objective of this study was to evaluate the feasibility of a Water-soluble Contrast Application into Dysphagia Evaluation	All patients >3 years old who were referred to for swallowing study from September 2015 to November 2017 at a tertiary medical center/university teaching hospital were recruited.  In total, 755 evaluations were analyzed, 365 of which were by WSS and 390 by MBSS.	Clinical trial: High-risk patients were evaluated by WSCA (iohexol)-based swallowing study (WSS) and others by MBSS. The specific dose is as follows:  Iohexol 350 (Omnipaque [osmolarity 541 mOsm/L, viscosity 10.4 centipoise at 37°C, specific gravity of 1.406],  Barium sulfate (BaSO4 40% [osmolarity 233 mOsm/L, viscosity 2.3 centipoise at 25°C])
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**Outcomes:**

The most common underlying condition was brain lesion, followed by aspiration pneumonia. Aspiration was more frequent in WSS (40.3%) compared to MBSS (9.2%). However, no significant differences were found between the groups in aspiration volume, radiographic alterations, or the correlation between iohexol intake and radiologic changes ( $p > .05$ ). Patients who underwent WSS more frequently transitioned to oral feeding (44.9%), with no increase in aspiration pneumonia. WSS did not delay discharge or cause allergic reactions or chemotoxicity within one week.

**Review:**

This study supports the use of WSC compared with MBSS because there were no aspiration-induced complications or adverse drug effects, along with increased sensitivity in detecting aspiration and facilitating an earlier transition to oral feeding.

**Table I: Summary of the articles reviewed**

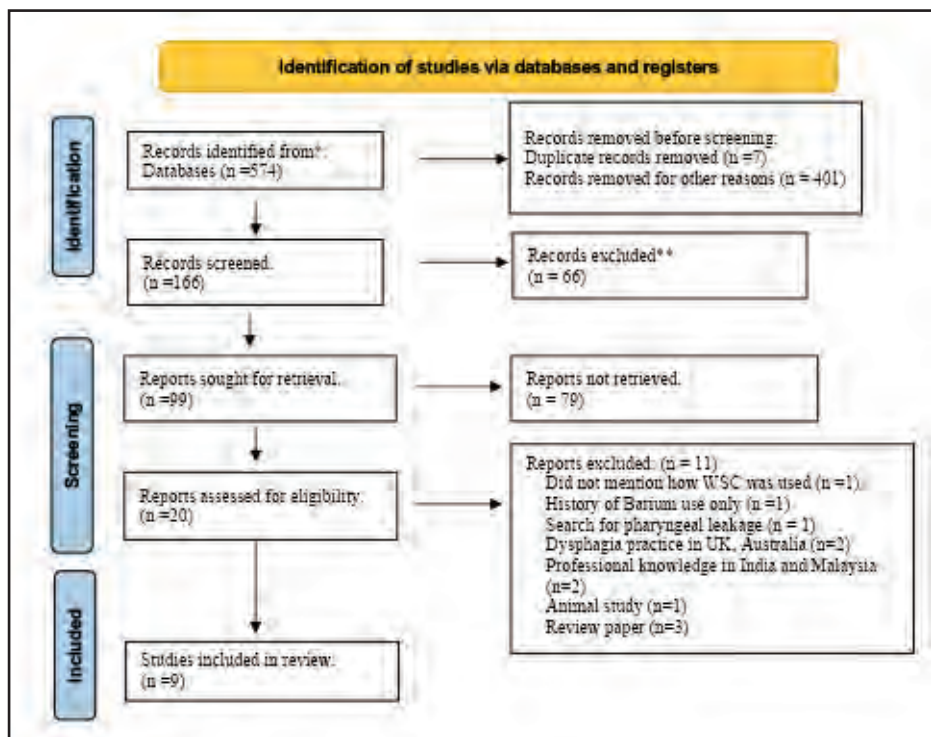
<p>The Returnus-2 study impact of respiratory muscle training in subacute stroke patients with dysphagia, study protocol of a double-blind randomized controlled trial</p>	<p>Authors: Guillen-Sola et al. Year: 2021 Country: Spain Study design: a double-masked randomized controlled trial</p>	<p>1) To evaluate the efficacy of an 8-week IEMT program on respiratory muscle strength and swallow dysfunction severity in patients who suffered from subacute stroke with dysphagia</p>	<p>Fifty stroke patients with oropharyngeal dysphagia.</p>	<p>Dysphagia severity was assessed using VFSS, FEES, the Penetration-Aspiration Scale, and the Bolus Residue Scale (BRS). 50 ml Visipaque Water-soluble contrast with a thickener (ThickenUp) in three different consistencies, liquid, nectar, and pudding, and three different volumes: 5-10- and 20 ml were used in VFSS. The strength of respiratory muscles was measured using PImax and PEmax. The voluntary peak cough flow (measured using a PEFR meter) and tongue strength (measured with the IOPI® system) were also measured. All data was collected at baseline, end-treatment, and 6-month follow-up.</p>
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**Outcomes:**

An improvement in respiratory muscle strength and might be associated with relevant benefits in dysphagia patterns. A reduction in aspiration events was confirmed by video-fluoroscopy or fiberoptic endoscopic evaluation of swallowing.

**Review:**

The results show a reduction in aspiration for those patients, which might be due to the effectiveness of the training program on muscles. However, the trial group's results or outcomes are not included yet.



**Fig. 1:** Prisma flowchart diagram of the screening process

found that the use of WSC in VFSS combined with surgical approaches such as supraglottic closure significantly improved swallowing function, reduced aspiration pneumonia risk, and enhanced overall quality of life.<sup>19</sup>

## DISCUSSION

This systematic review synthesizes findings from nine studies over the past decade, offering critical insights into the use of WSCs in VFSS. The discussion centers on the variability of WSC agents and their clinical applications and the implications for healthcare professionals, specifically radiologists and SLP's in the management of dysphagia.

### Variability of WSC agents in VFSS

Due to their safety and diagnostic efficacy, WSC agents are increasingly used in VFSS. The primary agents discussed in the reviewed studies include Iohexol, Omnipaque, Visipaque, and Gastrografin. These agents are preferred due to their lower risk of aspiration and ease of use than barium, particularly in patients at risk for aspiration pneumonia. Iohexol was commonly used in VFSS to evaluate pharyngeal and esophageal function in a large patient population.<sup>15</sup> Omnipaque (350 mg/mL) was used in Finland to investigate laryngeal penetration-aspiration in patients with spinal cord injuries.<sup>17</sup> Although WSCs have proven effective in detecting aspiration, clear guidelines on the optimal concentration and volume remain undefined.<sup>15</sup> Variability in WSC application is evident, as seen in a study exploring its use in head and neck surgical patients and esophageal assessments, which highlighted the benefits of low-osmolar agents such as Iohexol.<sup>20</sup> Their findings, alongside updates from the American expert panel in 2019, underscored the potential benefits of WSC, deficient osmolar variants like Iohexol.<sup>4</sup> Visipaque was employed in both pediatric and adult populations however, the concentration was not specified, and there was a noted lack of comparative analysis with other contrasts in pediatric patients.<sup>15</sup> Gastrografin (3-5 mL) was used in India in the Amrita Institute's Department of Head and Neck due to its lower reactivity with tissues in cases of extravasation to detect pharyngeal leaks post-laryngectomy.<sup>13</sup> While effective, the study did not address potential long-term complications associated with its use. Geographical differences in WSC use further illustrate the inconsistency in practice. For example, in Australia, the Western Australia Country Health Service recommended undiluted Iohexol (350 mg I/mL) in 50–100 mL volumes for thin fluid trials, which was tested in post-radiotherapy patients without adverse effects.<sup>11</sup> Similarly, a Korean study involving 762 patients reported no cases of pulmonary edema or aspiration pneumonia linked to Iohexol use complications typically associated with agents like Gastrografin.<sup>22</sup> The lack of standardized recommendations for concentration and volume continues to pose challenges for clinical practice.

### Comparison to Barium

While barium remains a standard contrast agent in VFSS, WSCs offer distinct advantages, particularly in patients at risk of aspiration. The usage of barium can cause significant complications if aspirated<sup>21</sup>, in contrast to WSC agents like Iohexol and Gastrografin, which are absorbed more readily

by the body, reducing the risk of severe complications.<sup>22</sup> However, the reviewed studies did not extensively compare WSC to barium in terms of diagnostic accuracy, patient outcomes, or safety profiles. This lack of direct comparison is a notable limitation; more studies are needed to reduce the ability to make informed decisions about the most appropriate contrast agent in different clinical scenarios.

### Implications for dysphagia management

The implications of using WSCs in VFSS extend beyond diagnostics to the management of dysphagia. As highlighted by this review, the use of WSCs in pediatric patients with neurological disabilities allowed for a more accurate diagnosis of dysphagia, leading to more individualized and effective treatment plans.<sup>15</sup> This is a critical consideration for SLP's who rely on precise diagnostic information to develop interventions that address the specific needs of their patients. Additionally, long term application of WSCs in VFSS, when combined with surgical interventions, has been associated with improved swallowing function in individuals experiencing chronic aspiration after radiotherapy for head and neck cancer.<sup>14</sup> These findings highlight the potential for WSCs to not only improve immediate diagnostic outcomes but also contribute to better long-term management of dysphagia, ultimately enhancing the quality of life for patients.

### Clinical Considerations and Recommendations

For healthcare professionals, particularly those in radiology and speech-language pathology, integrating WSCs into VFSS protocols presents both opportunities and challenges. The evidence supports using low-osmolar WSCs in specific patient populations, particularly those at high risk for aspiration or pulmonary complications. However, the choice of contrast agent should be guided by a thorough assessment of the patient's overall health, swallowing function, and specific risk factors. Given the benefits and safety profile of WSCs, it is recommended that clinicians consider these agents as part of a comprehensive VFSS approach, particularly in cases where barium sulfate may be contraindicated. Continued research is also necessary to further refine the use of WSCs in VFSS and to explore their application in broader patient populations.

### LIMITATIONS

This systematic review focuses on the application of WSC agents in VFSS examinations. There are several notable limitations in this review that impact the generalizability and strength of its findings. First, the inclusion of only nine studies may not fully capture the breadth of available evidence, limiting the overall representativeness. The studies analyzed also varied significantly in design, patient populations, and methodologies, leading to challenges in synthesizing results and drawing definitive conclusions. Additionally, the lack of standardized outcome measures across studies introduced variability, complicating the comparison of efficacy and safety outcomes. Potential publication bias, where studies with positive outcomes are more likely to be published, further skews the review's conclusions. Most studies focused on immediate rather than long-term outcomes, leaving a gap in understanding the

chronic implications of WSC use. Geographical and population-specific limitations may restrict the applicability of findings across diverse clinical settings. Moreover, several studies lacked critical methodological controls such as blinding and randomization, which could introduce bias and affect the reliability of results. Inconsistent reporting of adverse effects also limits the ability to fully assess the safety profile of WSCs. These limitations underscore the need for more rigorous, standardized research to validate the role of WSCs in VFSS better and improve clinical practice.

### RECOMMENDATIONS FOR FUTURE RESEARCH

Despite these advancements, a significant practice gap persists regarding the optimal concentration and volume of WSC. This gap underscores the need for standardized guidelines and protocols for using WSC in dysphagia assessments. Future research is essential to establish the most effective WSC concentrations for various food and liquid consistencies, thereby enhancing the efficacy and safety of dysphagia evaluations through VFSS.

### CONCLUSION

This systematic review shows that research into using WSC agents in VFSS is still emerging, with limited scientific and experimental studies available. Nevertheless, the current review suggests that WSC agents offers a viable alternative to Barium Sulfate in VFSS, particularly for patients at high risk of aspiration or penetration. There remains a lack of standardized guidelines regarding the type, concentration, and volume of WSC for optimal use in dysphagia evaluation. Further research is needed to establish definitive protocols to optimize the use of WSC in dysphagia evaluation and ensure consistent, safe practices across different clinical settings.

### CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors report no conflicts of interest in relation to this study. This research was self-funded by the authors. The first author would like to express his sincere gratitude to Zarqa University for the support and affiliation which make this work possible.

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# Impact of structured physical exercise during pregnancy on maternal health and fetal outcomes: A systematic review

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## ABSTRACT

**Introduction:** Current guidelines recommend that pregnant women engage in physical activity. Exercise during pregnancy has been shown to positively impact both maternal and fetal outcomes. Therefore, we conducted a systematic review to evaluate the impact of structured physical activity during pregnancy on maternal health and fetal outcomes.

**Materials and Methods:** A systematic search was conducted on relevant articles published between 2015 and 2020 using PubMed, Google Scholar, ScienceDirect, Scopus, and the Cochrane Library. Studies on pregnant women, comparative study designs with concurrent controls, structured physical intervention and health outcomes for both maternal and fetal were targeted. Maternal outcomes include gestational weight gain, lumbopelvic pain, gestational diabetes and pre-eclampsia, while fetal outcomes include prematurity and birth weight. Only original studies with published data were included. This review was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis.

**Results:** A total of 3441 published articles were retrieved from different databases. After assessing the obtained papers, studies that did not meet the inclusion criteria were excluded. Twenty studies involving 5188 populations that met the inclusion criteria were included in this review. However, due to the heterogeneity of the studies, meta-analysis was not done. Structured physical activity significantly reduced the risk of gestational diabetes (OR: 0.57; 95% CI: 0.40–0.81;  $p = 0.002$ ), but had no statistically significant effect on lumbopelvic pain (OR 0.98 (95% CI: 0.49 to 1.93)  $p=0.95$ ), gestational weight gain (OR 0.88 95% CI: (0.44, 1.76)  $p=0.71$ ), pre-eclampsia (OR 0.78, 95% CI: 0.41, 1.49,  $p=0.46$ ) causing preterm delivery (OR 0.91, 95% CI (0.69, 1.20),  $p=0.50$ ) or affecting fetal birth weight (mean difference 0.93, 95% CI (-42.67, 44.53),  $p=0.97$ )

**Conclusion:** Structured physical activity during pregnancy, three times a week, 30 minutes per day of moderate intensity, is safe and benefits pregnant women and the fetus. Our findings challenge the view that physical exercise during pregnancy is not helpful and can be harmful. To generate further evidence, there is a need for high-quality, standardised trials that assess specific types of structured

exercise program with better reporting of adherence and outcomes.

## KEYWORDS:

*Physical activity, lumbopelvic pain, pre-eclampsia, gestational diabetes, preterm, birthweight, pregnancy*

## INTRODUCTION

Physical activity is beneficial and vital in preventing non-communicable diseases, cancer, and other health conditions and improving overall well-being, including that of pregnant women. Despite its benefits, an estimated 1.4 billion adults are physically inactive. It is reported that inactive people have a 20% to 30% increased risk of death than those who are sufficiently active.<sup>1</sup>

Pregnancy represents a critical window for both maternal and fetal health, with physiological and metabolic changes that can significantly influence long-term outcomes. Among the most concerning complications during pregnancy are gestational diabetes mellitus (GDM), excessive gestational weight gain (GWG), pre-eclampsia and lumbopelvic pain, all of which are associated with adverse perinatal and postpartum outcomes.<sup>2</sup>

Physical activity has emerged as a promising, non-pharmacological intervention to mitigate pregnancy-related risks. Current international guidelines, including those from the World Health Organization (WHO, 2022) and the American College of Obstetricians and Gynecologists (ACOG, 2020), recommend that pregnant women without contraindications engage in at least 150 minutes of moderate-intensity physical activity per week before, during and after pregnancy.<sup>3</sup> The benefits of regular prenatal exercise may extend beyond physical fitness, potentially reducing the risk of GDM, managing weight gain within recommended limits, alleviating musculoskeletal discomfort, and enhancing mental well-being.<sup>2</sup>

Structured physical exercise refers to planned, organised, and repetitive physical activities designed to improve or maintain specific aspects of physical fitness, such as strength, endurance, flexibility, or cardiovascular health.<sup>4</sup> Unlike general physical activity, which includes any bodily movement that increases energy expenditure (e.g., walking

This article was accepted: 08 July 2025

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to work or household chores), structured exercise follows a defined regimen with set goals, intensity levels, and durations.

For example, structured physical exercise may include supervised training programs, aerobic workouts, resistance training, or high-intensity interval training (HIIT), often tailored to individual needs or health objectives. Research highlights its effectiveness in improving physical fitness and health outcomes, particularly when systematically prescribed based on factors like frequency, duration, and type of exercise.<sup>3</sup>

Previous studies have shown a positive impact of physical exercise on maternal health and fetal development. For example, exercise intervention has been proven to prevent excessive weight gain<sup>5,7</sup>, reduce the incidence of gestational diabetes<sup>5,7</sup> and pregnancy-induced hypertension<sup>8</sup>, and decrease the intensity of lower back pain and pelvic girdle pain.<sup>9,10</sup> Furthermore, exercise also helps reduce the total duration of labour,<sup>11</sup> and decrease the incidence of instrumental delivery.<sup>12</sup> In addition, more studies have shown that physical activity during pregnancy does not cause preterm birth or low birth weight.<sup>7</sup>

Studies have shown that most pregnant women are not physically active during pregnancy due to several factors, such as lack of energy or sickness due to pregnancy, lack of motivation, lack of time or busyness due to work and myths regarding exercise can cause miscarriage.<sup>13</sup> Nevertheless, the forms of exercise during pregnancy that benefit pregnancy, and maternal outcomes are still debatable.

Although several studies have investigated exercise during pregnancy, there is a lack of a comprehensive review that synthesizes the evidence on the impact and effect of structured physical activity on maternal and fetal outcomes. Hence, this systematic review aims to evaluate the effect and impact of structured physical activity on maternal health and fetal outcomes, particularly gestational diabetes, pre-eclampsia, gestational weight gain, lumbopelvic pain, birth weight, and preterm delivery. This review focuses on articles from 2015 onwards based on the establishment of ACOG guidelines on physical activity and exercise, which was published in 2015.

## MATERIALS AND METHODS

### Protocol and Registration

The present review was registered with the International Prospective Register of Systematic Reviews (PROSPERO) with clinical trial CRD42021229914 and conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).

### Search strategy

All authors developed and reviewed a search strategy (Box 1). Medical Subject Headings terms and keywords were used in various combinations. We searched published literature from five online databases: PubMed, Google Scholar, Science Direct, Scopus, and Cochrane Library databases to identify studies examining the effect of the type of structured exercise

program during pregnancy on maternal and fetal health outcomes. We conducted the search in May 2021.

Box 1. Search strategy for impact of exercise on maternal and fetal health

1. Effect OR Impact
2. Structured OR Supervised
3. Physical exercise OR exercise
4. Pregnancy OR pregnant women OR gestation
5. #1 AND #2 AND #3 AND #4

### Inclusion criteria

We selected all Randomized Controlled Trials (RCTs) worldwide involving a structured plan on physical activity that reported the effect of structured physical exercise on maternal and fetal health outcomes and fulfilled the following criteria. The criteria included English peer review articles published from January 2015 to December 2020 due to the establishment of ACOG guidelines on physical activity and exercise, which was published in 2015, studies involving pregnant women with singleton pregnancies and low-risk and uncomplicated pregnancy. Articles for which the full text could not be obtained after two email contacts from the principal investigator and quasi-experimental studies were excluded. In addition, conference abstracts and grey literature were excluded because they may not be subjected to the same peer review rigour.

### Study selection

We imported relevant articles identified through the databases into a Microsoft Excel Spreadsheet and removed duplicate publications. Two reviewers independently performed the screening using the titles and abstracts to search for potentially eligible articles based on the inclusion and exclusion criteria mentioned above. Discussions were held to resolve any disagreement with a final consensus before reviewing the full text of each relevant article.

### Data Extraction

Two review authors (FHMN and NAJ) screened the titles and abstracts independently to identify potentially relevant citations. These review authors retrieved the full texts of all potentially relevant articles and independently assessed the eligibility of the studies using predefined inclusion criteria. We extracted data from all the articles found to be relevant. The authors of the primary studies were contacted by email in case more complete information was needed. Any disagreements or discrepancies between reviewers were resolved by discussion and, if necessary, consulting a third author (KHAA). In addition to the electronic search, we searched for reference lists of the articles identified.

### Quality assessment

We evaluated the quality of the studies using the risk of bias by the Cochrane Collaboration tool.<sup>14</sup> It covers six domains of bias: selection bias, performance bias, detection bias, attrition bias, reporting bias, and other biases. Within each domain, assessments are made for one or more items, which may cover different aspects of the domain or different outcomes.

### Data synthesis

We analyzed the maternal and fetal outcomes among pregnant women who participated in a structured exercise program compared to those in the control groups. Statistical analysis was conducted using Review Manager (RevMan) software version 5.4. Only published data were used. No attempts were made to retrieve raw data from the original study authors.

Odd ratios (OR) or mean differences (MDs) were calculated with 95% confidence intervals using the Mantel-Haenszel method. A fixed effects model was applied. Heterogeneity was assessed with I<sup>2</sup> statistics, and a random effects model would have been used if significant heterogeneity was detected (I<sup>2</sup>>50%).

The ORs were reported for dichotomous data (excessive gestational weight gain, gestational diabetes, pre-eclampsia, lumbopelvic pain intensity, and preterm delivery). In contrast, MDs were reported for the continuous outcome of birth weight.

A meta-analysis was not conducted due to substantial clinical and methodological heterogeneity across the included studies, notably variations in participants' gestational age, differences in study quality and varying risk of bias.

## RESULTS

### Characteristics of the studies

A total of 3441 studies were retrieved from different databases. After removing the duplicate articles (n=814) and reading the titles and abstracts, articles not fulfilling the inclusion criteria were excluded. A total of 33 full-text articles were downloaded, and their eligibility was assessed. Of these articles, 13 were excluded because they did not meet the inclusion criteria (among the ten papers, the outcome of interest was not reported, and the other three papers were not primary studies). Finally, 20 articles were used for this systematic review. The detailed selection procedure, as outlined in the PRISMA guidelines, is illustrated in Figure 1.

The summary characteristic of the included studies is listed in Table I. Fourteen (14) studies were conducted in Europe; nine were in Spain<sup>15-22</sup>, two in Norway<sup>23-24</sup>, and each one in Sweden<sup>25</sup>, Denmark<sup>26</sup>, and France.<sup>27</sup> The rest of the studies were conducted: one in Brazil<sup>28</sup>, Colombia<sup>29</sup>, Slovenia<sup>30</sup>, Iran<sup>31</sup>, Nigeria<sup>32</sup>, and Australia<sup>33</sup>. A total of 5,179 participants were involved in the studies, comprising 2,378 pregnant women in the exercise group and 2,801 participants in the control group. The majority of the exercise took place in hospitals or medical centre<sup>15,16,31,32,34,17,19,20,22,23,27,29,30</sup>, three in universities,<sup>18,26,28</sup> two at public facilities<sup>21,24</sup> and one at the clinic.<sup>25</sup> For the studies, the exercise sessions were conducted by a physiotherapist-supervised exercise session<sup>17,22,25,30,32,33</sup>, an exercise instructor<sup>16,19,23,24,26,28,31,34</sup>, fitness instructors<sup>15,18,20</sup>, gynaecologist<sup>27</sup> or dietician<sup>29</sup>. All women in the control group were given standard antenatal care.

### Type of structured physical exercise

The types of exercise varied among the studies. Most studies have conducted aerobic exercises<sup>15,16,30,32,33,17,19,20,22-24,28,29</sup>, three studies performed aquatic exercises<sup>18,21,26</sup>, and each one conducted yoga<sup>31</sup>, Pilates<sup>34</sup>, and resistance exercises.<sup>25</sup> Meanwhile, Cordero et al., 2015<sup>18</sup> conducted mixed land-based and aquatic activities. Most of the intensity levels were moderate, except for three studies that had light to moderate intensity.<sup>17,20,27</sup> and there were two studies on vigorous intensity.<sup>25,29</sup> These categories were established according to the Borg scale,<sup>21,23,28,32</sup> with the scoring of perceived exertion, heart rate<sup>17,22,29</sup>, the combination of the Borg scale and heart rate<sup>15-20,30,33</sup>, and a questionnaire to assess metabolic equivalents of task (METs).<sup>24</sup> Five studies did not mention the indications of the intensity of the exercise.<sup>25-27,31,34</sup> The exercise groups were done at least twice to three times per week, ranging from 45 to 65 min. Most of these studies were conducted in the first or second trimester until delivery, except for a study by Sklempekovic et al.<sup>30</sup>, which started in the third trimester.

### Risk of bias

Risk of bias was assessed using the Cochrane Risk of Bias Tool, as implemented in Review Manager (RevMan). Two independent reviewers (FHMN, NAJ) performed the assessment. Disagreements were resolved through discussion or consulting a third reviewer (KHAA).

The following seven domains were evaluated: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other sources of bias. Based on the information reported in the individual studies, each domain was judged as having a 'low risk,' 'high risk,' or 'unclear risk' of bias. Figures II and III present a graphical summary of the risk of bias across all included studies (generated via RevMan). Figure II illustrates the quality assessment regarding random sequence generation. Nine studies lacked sufficient data on the randomised process, so we assessed the risk of bias as unclear.<sup>15,18,20,23,25-27,30,32</sup> One study randomised participants based on volunteerism, which we evaluated as a high risk of bias.<sup>25</sup>

Most studies used numbered, opaque, and sealed envelopes to conceal the allocation, which we considered to be a low risk of bias. However, nine studies were assessed as having an unclear risk of bias due to insufficient methodological details on allocation concealment.<sup>18,19,21,22,27,29,30,32,33</sup> All twenty studies reported that participants were blinded; therefore, the risk of bias was assessed as low. However, eleven studies did not specify whether the outcome assessors were blinded. Despite attempts to confirm this with the authors, we concluded these studies as having an unclear risk of bias.<sup>18-20,22,26,27,29,31-34</sup> One study was judged to be at high risk of bias because the researchers were not blinded.<sup>21</sup>

Approximately 65% of the studies analysed all enrolled participants, resulting in a low risk of bias. Seven studies were

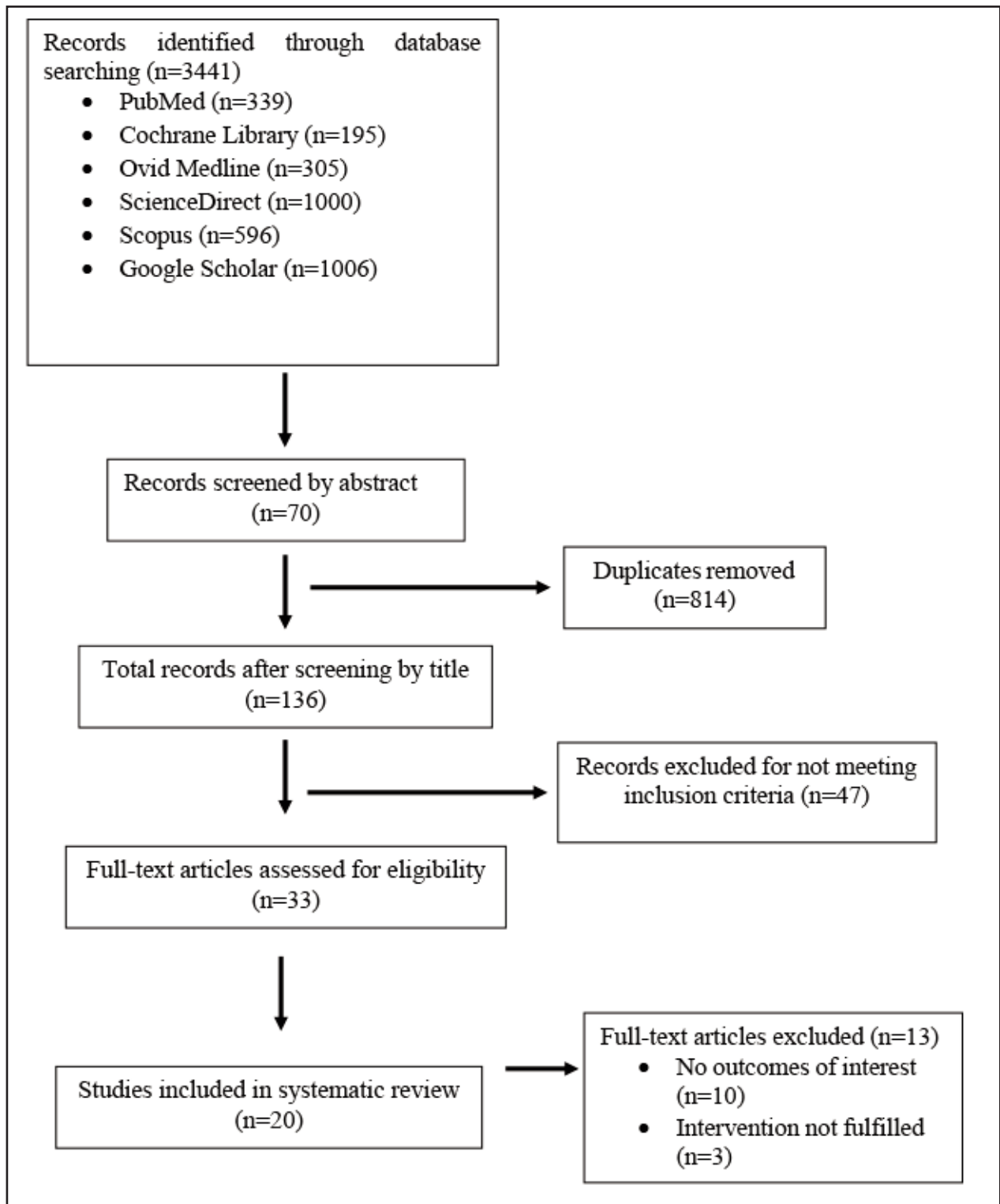


Fig. 1: PRISMA flow diagram of literature search for the effects of structured physical exercise during pregnancy

judged to have a high risk of bias due to inadequate reporting of missing data and the inclusion of participants who dropped out of the intervention group.<sup>17,20,22,25,28,29,32</sup> One study had an unclear risk of bias because it presented findings without providing absolute values for pregnant women in the exercise and control groups.<sup>26</sup>

Regarding other biases, three studies were assessed as having an unclear risk of bias. In one study<sup>21</sup>, although an intention-to-treat analysis was stated to have been used, not all participants were included in the analysis. Two studies did not report the participants' baseline characteristics.<sup>18,22</sup>

Table 1: Characteristics of included studies on structured physical activity and the outcome

Authors/ year	Subject		Intervention description					Outcomes	
	IG	CG	Type of exercise	Duration (min)	Intensity	Frequency (times/week)	Gestational period (weeks)	Maternal	Fetal
Daniel 2015	35	35	Aerobic	45-60	Moderate	2	From 16 weeks until the week of delivery	Exercise programs in pregnant women did not increase the risk of preterm labour. However, the gestation duration was significantly longer in the exercise group than in the control t (68) = 2.315; p = 0.026).	No statistical difference in birth weight (EG 3.37kg; CG 3.31kg; MD 60.00; 95% CI -252.28;372.28; p=0.72).
Cordero 2015	122	220	Aquatic water-based activity	50-60	Moderate	3	From 10-14 weeks until 36 weeks	Maternal exercise using aerobic and muscular conditioning on land and in the water with high compliance reduced the incidence of GDM (OR 0.10; 95% CI 0.013,0.803; p=0.009). It was associated with a decrease in gestational weight gain (OR 0.56; 95% CI 0.31,0.99; p=0.04).	No statistical difference in birth weight (mean difference EG 3324.1 ± 433.1; CG 3250.1± 425.01; MD 74.00, 95% CI -33.62,181.62; p=0.177).
Haakstad 2015	52	53	Aerobic	60	Moderate	At least 2	From 24 weeks to 36 weeks	There were no statistical differences between the exercisers and controls in numbers reporting low back pain after the intervention (OR = 1.10; CI 0.47–2.60; p=0.83).	
Fieril 2015	51	41	Resistance	60	Moderate-vigorous	2	From 14 weeks until 28 weeks		<b>Birth weight</b> Newborns delivered by women who underwent resistance exercise during pregnancy were significantly heavier than those born to control women (EG 3561±452g; CG 3251±437g; MD 310.00; 95%CI 104.50, 515.50; p= 0.02), a difference that disappeared when gestational age was adjusted (p = 0.059).
Barakat 2016	420	420	Aerobic	40	Moderate	3	From 9-11 weeks until 38-39 weeks	<b>Pre-eclampsia</b> The exercise intervention reduced the incidence of pre-eclampsia (0.5% vs 2.3%; OR 0.22; 95% CI 0.05, 1.02; p=0.03). <b>Gestational weight gain</b> Pregnant women who did not exercise were 1.5 times more likely to gain excessive weight (OR 1.47; 95% CI 1.06, 2.03; p= 0.02). <b>Gestational diabetes</b> Exercise reduced the incidence of GDM (2.4% vs 5.5%; OR 2.05; 95% CI 0.91, 4.6; p=0.03).	<b>Birth weight</b> Pregnant women who do not exercise were 2.5 times more likely to give birth to a macrosomic infant (OR, 2.53; 95% CI 1.03, 6.20; p=0.04).

Table 1: Characteristics of included studies on structured physical activity and the outcome (Continued)

Authors/ year	Subject		Intervention description					Outcomes	
	IG	CG	Type of exercise	Duration (min)	Intensity	Frequency (times/week)	Gestational period (weeks)	Maternal	Fetal
Guelfi 2016	85	87	Pedalling cycling	60	Moderate	3	From 14 weeks until delivery	The supervised home-based exercise started at 14 weeks of gestation did not prevent the recurrence of GDM (OR 1.02; 95% CI 0.55, 1.89; p=0.95). No statistical difference in the: - Pre-eclampsia (OR 2.05; 95% CI 0.18, 23.03; p=1.000)	No statistical difference in the: - Preterm delivery (OR 0.75; 95% CI 0.16, 3.46; p=1.000) - Birth weight (EG 3552 ± 469g; CG 3419± 518g; MD 133.00; 95% CI; -16.04,282.04; p=0.82)
Jahdi 2016	30	30	Supervised yoga	60	Moderate	3	From 26 weeks until 37 weeks		No statistical differences in birth weight (EG 3005.6 ± 176.5g; CG 3156.6 ± 469.1g; MD -151.00; 95% CI -330.35, 28.35; p=1.0).
Perales 2016	122	117	Aerobic	55-60	Light-moderate	3	From 9-11 weeks until 39-40 weeks	The groups did not differ in - Total gestational weight gain (OR 0.47; 95% CI 0.22, 0.99; p=0.06)	The groups did not differ in - Birth weight (EG 3183.6 ± 446.8g; CG 3232.1 ± 383.2g; MD -48.50; 95% CI -175.13, 78.13; p=0.46)
Ramirez- Velez 2017	24	26	Aerobic, resistance	60	Moderate-vigorous	3	From 16-20 weeks until 28-34 weeks	Lumbopelvic pain Low back pain intensity was 0.38 (95% CI 0.02-0.74); p=0.04) lower in the water exercise group.	There was no significant difference between the exercise and control groups regarding mean birth weight (EG 3133 ± 406g; CG 3013 ± 494g; MD 120.00; 95% CI -129.88, 369.88; p=0.34).
Backhausen 2017	258	258	Water exercises	45	Moderate	2	From 20 weeks until 32 weeks	There was no difference in: Pre-eclampsia (OR 0.83; 95% CI 0.30, 2.34; p=0.57) - gestational diabetes (OR 0.96; 95% CI 0.06, 15.41)	There was no difference in: - Preterm delivery (OR 0.56; 95% CI 0.20, 1.58; p=0.34) - Birth weight (EG 3549 ± 531g ; CG 3540 ± 531g; MD 9.00 ; 95% CI -87.03, 105.03; p=0.66)
Rodriguez-Diaz 2017	50	53	Pilates method	40-45	Moderate	2	From 26-28 weeks until 34-36 weeks		Eight weeks of the Pilates program did not cause an adverse effect on birth weight (EG 336.23 ± 361.88; CG 3417.6 ± 473.54; MD -55.37; 95%CI -221.57, 108.83; p=0.005).
Sklempekovic 2017	22	23	Aerobic, resistance	50-55	Moderate	2	From 30 weeks until 36 weeks	The exercise program had a beneficial effect on the severity of lumbopelvic pain in pregnancy as it reduced the intensity of the pain (the numeric rating scale PGQ (p=0.017) and RMDQ score (p=0.005) in the 36th week of pregnancy.	

Table 1: Characteristics of included studies on structured physical activity and the outcome (Continued)

Authors/ year	Subject		Intervention description				Outcomes		
	IG	CG	Type of exercise	Duration (min)	Intensity	Frequency (times/week)	Gestational period (weeks)	Maternal	Fetal
Watelain 2017	45	45	Muscle strengthening	60	Light-moderate	2	From 24 weeks until 36 weeks	Strengthening exercises centred on the trunk reduce low back pain intensity (p<0.0001).	
Sanda 2018	303	303	Cardiovascular	60	Moderate	2	From mean gestational week 17.6 ±2.6 until delivery (mean gestational week 39.9 ±1.8)		No statistical differences in - Preterm delivery (OR 1.00; 95% CI 0.50, 1.99; p=0.83) - Birth weight (EG 3410.6 ± 486.2g; CG 3449.7 ± 539.3g; MD -39.10; 95% CI -122.04, 43.84; p=0.36)
Brik 2019	42	43	Cardiovascular	60	Light-moderate	3	From 9 weeks until 38 weeks	<b>Gestational weight gain</b> No difference in maternal weight was measured at 20, 28, 36, and 38 weeks gestation or in weight gain at 38 weeks between women who followed the exercise program and those who did not (OR 0.86 95%; CI 0.26, 2.80; p=0.51).	No significant difference in the - Preterm birth (OR 0.50; 95% CI 0.04, 5.73; p=0.66) - Birth weight (EG 3161 ± 564.4g; CG 3477.11 ± 414.51g; MD -40.00; 95% CI -242.68, 162.68; p=0.36)
Barakat 2019	260	260	Aerobic	55-60	Moderate	3	From 8-10 weeks until 38-39 weeks	Gestational weight gain Exercise throughout pregnancy can reduce the risk of excessive maternal weight gain (OR 0.60; 95% CI 0.39, 0.92; p=0.018). Gestational diabetes the prevalence of gestational diabetes was significantly higher in the CG than the EG (6.8% vs 2.6% respectively; OR 0.363; 95% CI 0.138,0.953; p = 0.033).	<b>Birth weight</b> No differences were found in birth weight between study groups (p=0.6). The ratio of neonate macrosomic was slightly higher in the control group than in the intervention group (7.2% vs 3.4%; OR 0.456; 95% CI, 0.191,1.087).
Rodriguez 2019	65	64	Aquatic aerobic exercises	60	Moderate	3	From 20 weeks until 37 weeks		The median birth weight was 3259g in EG and 3477 grams in IG, and the difference was statistically significant (MD -218.1; 95% CI - 388.81, -47.41; p=0.011).
Roldan- Reoyo 2019	64	67	Aerobic	60	Moderate	3	From 10-12 weeks until 36-40 weeks		No statistical difference in birth weight (EG 3139 ±451.52g; CG 3222.41 ± 416.62g; MD - 83.41; 95% CI -273.65, 106.83; p>0.005).

Table 1: Characteristics of included studies on structured physical activity and the outcome (Continued)

Authors/ year	Subject		Intervention description				Outcomes		
	IG	CG	Type of exercise	Duration (min)	Intensity	Frequency (times/ week)	Gestational period (weeks)	Maternal	Fetal
Pelaez 2019	115	230	Aerobic, resistance	60-65	Moderate	3	From 12 weeks until 36 weeks	Supervised moderate to vigorous exercise performed throughout gestation was influential in preventing EGW gain (22 [22.0%] vs 69 [34.3%]; OR 0.54; 95% CI 0.31, 0.04; p=.03). It also prevented gestational diabetes (OR 0.45; 95% CI 0.12, 1.61; p=0.03)	
Da Silva 2017	213	426	Aerobic	60	Moderate	3	From 16-20 weeks to 32-36 weeks	No differences between the two groups in - mean gestational weight gain (OR 1.03; 95% CI 0.70, 1.52; p=0.10) - pre-eclampsia (OR 0.99; 95% CI 0.47, 2.09; p=1.0) - gestational diabetes (OR 1.03; 95% CI 0.55, 1.92; p=1.0)	No differences between the two groups in - birth weight (EG 3234 ± 511g; CG 3254 ± 467g; MD -20.00; 95% CI -103.52, 63.52; p=0.63). The exercise program did not cause adverse impacts on preterm birth (OR 1.10; 95% CI 0.66, 1.83; p=1.1)

## Maternal Health Outcomes

### Gestational weight gain

Seven randomised controlled trials were analysed to evaluate the effect of maternal exercise on gestational weight gain, as shown in Figure IV. The pooled analysis included 2,218 participants, with 961 in the exercise group and 1,257 in the control group. The overall odds ratio (OR) for excessive gestational weight gain in the exercise group compared to the control group was 0.88 (95% CI: 0.44 to 1.76), indicating a non-significant risk reduction ( $Z = 0.37$ ,  $p = 0.71$ ). While several studies demonstrated a favorable trend toward reduced weight gain with exercise, the confidence intervals often overlapped the line of no effect. Notably, substantial heterogeneity was observed across studies ( $\text{Tau}^2 = 0.76$ ;  $\text{Chi}^2 = 62.12$ ,  $df = 6$ ,  $p < 0.00001$ ;  $I^2 = 90\%$ ), likely reflecting differences in study populations, exercise protocols, and outcome definitions. Despite the lack of statistical significance in the pooled estimate, the direction of effect suggests a potential benefit of maternal exercise, warranting further investigation through well-designed, homogeneous trials.

### Lumbopelvic pain

Four studies were identified that reported on lumbopelvic pain (LBP), measuring pain intensity level<sup>26,27,30</sup> and prevalence of LBP among pregnant women.<sup>23</sup> Out of these studies, one study reported no statistically significant results.<sup>23</sup> Three randomised controlled trials were analysed to assess the effectiveness of exercise in reducing lumbopelvic pain during pregnancy, as shown in Figure V. The analysis included a total of 753 participants (375 in the exercise group and 378 in the control group). The pooled odds ratio (OR) was 0.98 (95% CI: 0.49 to 1.93), indicating no statistically significant difference in the odds of experiencing lumbopelvic pain between the exercise and control groups ( $Z = 0.06$ ,  $p = 0.95$ ). The test for heterogeneity revealed no significant variation across studies ( $X^2 = 0.63$ ,  $df = 2$ ,  $p = 0.73$ ;  $I^2 = 0\%$ ), indicating consistent findings. While individual study estimates varied, none demonstrated a significant benefit of exercise. One study could not be included in the meta-analysis due to non-estimable data.<sup>27</sup> Overall, the findings suggest that exercise may not significantly impact the prevention or reduction of lumbopelvic pain in pregnancy. However, the wide confidence intervals and limited number of studies indicate that further high-quality trials are warranted.

### Gestational Diabetes

Seven randomised controlled trials were analysed to evaluate the effect of maternal exercise during pregnancy on the risk of developing gestational diabetes mellitus (GDM), as shown in Figure VI. Two studies used the American Diabetes Association (ADA) criteria,<sup>18,19</sup> and one study was based on the International Association of Diabetes and Pregnancy Study Groups (IADPSG),<sup>33</sup>. Four other studies did not specify their diagnostic criteria.<sup>15,16,26,28</sup> Four studies reported statistically significant results.<sup>16,18,19,35</sup>

The pooled data comprised 2,887 participants, consisting of 1,222 women in the exercise group and 1,665 in the control group. The analysis yielded a statistically significant reduction in the odds of GDM among women who engaged in exercise, with a pooled odds ratio (OR) of 0.57 (95% CI: 0.40 to 0.81;  $Z = 3.10$ ,  $p = 0.002$ ). The findings suggest a 43%

lower risk of GDM in the exercise group than in controls. Heterogeneity among the studies was low ( $\text{Chi}^2 = 8.53$ ,  $df = 6$ ,  $p = 0.20$ ;  $I^2 = 30\%$ ), indicating consistency across the included trials. Several studies, including those by Barakat (2016, 2019) and Cordero (2015), reported significant protective effects of exercise.<sup>15,16,18</sup> The results support the role of prenatal exercise as an effective intervention for reducing the incidence of GDM, reinforcing current public health recommendations promoting physical activity during pregnancy.

### Pre-eclampsia

Figure VII shows the analysis that explores the impact of exercise on the risk of pre-eclampsia, synthesising data from four studies published between 2016 and 2017. The included studies span a total of 2,062 participants, comparing exercise interventions to control groups. The cumulative analysis revealed an odds ratio (OR) of 0.78 (95% CI: 0.46–1.32), suggesting a potential reduction in pre-eclampsia risk among individuals who engage in exercise, although the result did not achieve statistical significance ( $P = 0.35$ ). The heterogeneity among the studies was low ( $I^2 = 19\%$ ,  $P = 0.30$ ), indicating consistency across findings. The most pronounced benefit was observed in the study by Barakat et al. (2016)<sup>15</sup>, which reported an OR of 0.22 (95% CI: 0.05–1.02). Despite these promising trends, the results highlight the need for further large-scale, randomised controlled trials to confirm the protective effects of exercise on pre-eclampsia, particularly in diverse populations.

### Fetal health Outcome

#### Premature delivery

Figure VIII shows the analysis that evaluates the impact of exercise on preterm delivery, incorporating data from seven studies conducted between 2016 and 2019.<sup>15,16,24,26,28,29,32,33</sup> The pooled analysis included 2,648 participants, comparing preterm delivery outcomes between exercise intervention groups and control groups. While the overall odds ratio (OR) of 0.80 (95% CI: 0.56–1.12) indicates a trend toward reduced risk with exercise, the association was not statistically significant ( $P = 0.19$ ). The analysis demonstrated low heterogeneity ( $I^2 = 0\%$ ,  $P = 0.94$ ), suggesting consistency across studies. The study by Barakat et al. (2016)<sup>15</sup> contributed the most weight (46.4%) to the analysis, with an OR of 0.87 (95% CI: 0.46–1.28). These results underscore the potential benefits of exercise in reducing preterm delivery, but highlight the necessity for further high-quality, randomised controlled trials to confirm these effects and elucidate the mechanisms.

#### Birthweight

Analysis from 16 studies conducted between 2015 and 2019 investigates the effect of exercise during pregnancy on infant birthweight as shown in Figure IX. The pooled analysis, encompassing a total of 3,681 participants, compared birth weight outcomes between the exercise and control groups. The overall mean difference (MD) of 7.84 grams (95% CI: -22.69 to 38.36) suggests a negligible effect of exercise on birthweight, with statistical insignificance ( $P = 0.61$ ). Moderate heterogeneity ( $I^2 = 49\%$ ,  $P = 0.02$ ) is observed, indicating variation across study findings. The most significant contribution to the analysis was observed in the study by Ramirez-Velez et al. (2017), which reported a MD of

-340.60 grams (95% CI: -544.00 to -137.20). These results suggest that while exercise during pregnancy does not substantially influence birth weight, further research is warranted to examine specific contexts and subpopulations where exercise may have significant impacts.

## DISCUSSION

This systematic review evaluated the impact of structured exercise on gestational outcomes among pregnant women, mainly gestational diabetes mellitus (GDM), gestational weight gain (GWG), lumbopelvic pain, pre-eclampsia, preterm delivery, and fetal birth weight. The exercise programs in the intervention group combined aerobic exercise, dance, resistance training, aquatic workouts, cardiovascular workouts, and Pilates, which were easily incorporated into the structured exercise regimen. Most of the studies were liked by pregnant women, as indicated by the high adherence rate.

Our findings suggest that structured physical activity during pregnancy significantly reduced the incidence of gestational diabetes by 40% compared to the control group. These findings are aligned with a meta-analysis by Sanabria-Martinez et al.<sup>36</sup> A meta-analysis involving 2,873 pregnant women from 13 studies found that structured physical exercise during pregnancy reduced the incidence of gestational diabetes by 31%. The impact was seen as greater when the exercise program was initiated during early pregnancy. Nasiri-Amiri et al. (2019), evaluated the effect of exercise on 1441 pregnant women in eight studies.<sup>54</sup> They reviewed that exercise decreased the risk of GDM in obese and overweight pregnant women. However, Davenport et al., (2018) found that antenatal exercise was ineffective in reducing the incidence of GDM.<sup>40</sup> This could be because unsupervised exercise leads to poor compliance with the exercise protocol, which in turn influences the results. Nevertheless, this finding may support that a healthy lifestyle initiated pre-conception and in the early trimester may be a fundamental method in preventing chronic disease risk in pregnant women.

Our review found that exercise intervention during low-risk pregnancy did not significantly impact the prevention of excessive weight gain. Still, considering the OR, exercise had a 25% protective effect in preventing excessive weight gain. Our findings aligned with a review and meta-analysis study by Shieh et al. (2018) concluded that physical exercise is ineffective in preventing excessive weight gain during pregnancy.<sup>37</sup> In addition, Kunath et al. (2019) demonstrated that combining physical exercise and diet was ineffective in avoiding excessive weight gain.<sup>38</sup>

In contrast, Wang et al., (2019) in a meta-analysis study found that active pregnant women in all BMI categories significantly gained less weight than the control group.<sup>39</sup> Furthermore, another review by Sui et al. (2012), which examined the effects of exercise among 276 overweight and obese pregnant women, found that supervised antenatal exercise lowered the risk of excessive weight gain. The authors also concluded that the impact of limiting gestational weight gain is more significant in the combination of exercise and diet groups.<sup>6</sup>

Structured physical activity has been associated with reduced risk of developing pre-eclampsia. Our review found that structured antenatal exercise lowered the risk of developing pre-eclampsia by 22% even though it was not statistically significant. Davenport et al. (2018) in their meta-analysis evaluated the effect of structured physical activity on 273,182 pregnant women in 106 studies and found that exercise reduced the risk of gestational hypertension by 39% and pre-eclampsia by 41%. The authors suggest following ACOG recommendations to achieve at least a 25% reduction in the odds of developing pre-eclampsia.<sup>12</sup> However, a descriptive study by Babili et al., (2021) reported that moderate to vigorous antenatal exercise did not reduce the incidence of pre-eclampsia.<sup>41</sup> Exercise performed during pregnancy was hypothesised to suppress peripheral insulin resistance, reduce oxidative stress, and promote placental growth and vascular development.<sup>42-44</sup> This mechanism reduces blood glucose and controls blood pressure.

Our systematic review also found that structured physical activity during antenatal did not reduce lumbopelvic pain (LBP) intensity or prevalence. Despite this, our review suggests that antenatal exercise improves the quality of life and disability caused by LBP. Shiri et al. (2018) evaluated the association between physical activity during pregnancy and LBP, which found that exercise intervention had no protective effect on LBP or pelvic girdle pain. However, they found that pregnant women taking sick leave due to LBP were less compared to the control group.<sup>9</sup> This could be explained by the fact that exercise improves muscle strength, lowers stress on the spine, and increases joint stabilization.<sup>10</sup> In contrast, a protective effect of exercise against LBP was seen in a few studies. A meta-analysis by Kinser et al. (2017) demonstrated that exercise during pregnancy reduced LBP, discomfort, and related symptoms.<sup>55</sup> Furthermore, a meta-analysis by Davenport et al. (2018) also found that exercise performed during pregnancy and the early postpartum period did reduce the severity of LBP compared to those who did not exercise.<sup>12</sup>

Our review also found that exercise during pregnancy does not affect birth weight and gestational length. Women with a higher exercise energy expenditure during pregnancy delivered infants with appropriate gestational age (AGA) birth weight without evidence of an increased risk for small or large gestational age (SGA/LGA). Betham et al., (2019) in their review examining the effect of vigorous physical activity on 32080 pregnant women in 13 studies, found that the exercise program did not increase the risk of delivering an SGA infant and reduced the risk of prematurity.<sup>45</sup> Moreover, our study and others suggest a reduction in the risk of delivering LGA infants with antenatal exercise.<sup>46-48</sup> In contrast, a case-control study by Mahmoodi et al., (2013) found that sports participation was linked to a greater risk of LBW infants.<sup>49</sup>

We also found no relationship between structured physical activity during pregnancy and the risk of prematurity. In contrast to a review by Aune et al. (2017), 20 RCTs and 20 cohort studies found that a high intensity of physical exercise during antenatal care decreased the risk of preterm birth by 14%.<sup>50</sup> Similarly, Di Mascio et al., (2016) showed that pregnant women can safely perform aerobic exercise as it

was not related to an increased risk of preterm birth.<sup>51</sup> Exercise increases insulin sensitivity and decreases the inflammatory process which may contribute to reducing the risk of preterm birth.<sup>52</sup> However, previous studies showed inconsistent findings, as they reported inverse associations between physical activity during pregnancy and preterm birth.<sup>52-53</sup> Takami et al. (2018) conducted a cohort study that showed physical activity during pregnancy increased the risk of preterm birth.

The findings of this review have important implications for clinical practice, particularly in the context of routine antenatal care. Given the observed reduction in the risk of gestational diabetes mellitus (GDM) among women who engaged in prenatal structured exercise, healthcare providers should proactively promote physical activity as a standard component of pregnancy care. Clinical guidelines recommend at least 150 minutes of moderate-intensity exercise per week during pregnancy for women without contraindications.<sup>3</sup>

Providers—obstetricians, family physicians, and midwives—should offer individualized exercise counselling, considering gestational age, medical history, and physical limitations. Structured programs, such as supervised group sessions or home-based regimens with follow-up, may improve adherence and outcomes.<sup>12</sup> Although the evidence for reducing lumbopelvic pain, gestational weight gain, pre-eclampsia, premature delivery and low birth weight remains inconclusive, the overall maternal benefits—ranging from improved glycemic control to enhanced psychological well-being—support the broader integration of exercise into antenatal pathways. Integrating exercise promotion into routine antenatal visits, supported by patient education and local resources, could improve pregnancy outcomes and long-term maternal health.

The limitations of our study include the fact that only randomized controlled studies, and English articles were included in our review. We excluded quasi-experimental studies or literature reviews. Additionally, we were unable to investigate the effect of specific exercise types on pregnancy outcomes due to differences in exercise type, the use of various exercises in some studies, and variations in exercise intensity and duration. Moreover, the variation in exercise duration across studies may impact the outcomes of this review. Another limitation was the inconsistency in the quality of evidence across the included studies, which could impact the results.

## CONCLUSION

Structured physical activity of moderate intensity, at least 150 minutes per week, can be safely performed during the antenatal period. It significantly reduces the overall incidence of GDM and is not associated with an increased risk of preterm birth or affecting birth weight. Our review also found that structured physical activity among uncomplicated pregnant women reduced the incidence of pre-eclampsia, prevented excessive weight gain, and improved LBP pain, even though it was not statistically significant.

However, most studies were based on various types, intensities, or durations of exercise and were inconsistent in quality. Hence, more trials are needed to find the effect of structured physical activity during pregnancy on gestational outcomes. As this review only includes randomised controlled trials, further study must be conducted to arrive at a complete conclusion on the impact of structured physical activity on pregnancy outcomes.

## SOURCE OF FUNDING

This research did not receive funding from any external sources.

## ACKNOWLEDGEMENT

The authors also thank the International Islamic University Malaysia and the Ministry of Health Malaysia for their approval and publication of our research.

## CONFLICT OF INTEREST

There is no conflict of interest.

## ETHICAL APPROVAL

This study was approved by the Research and Ethical Committee of researchers' institution (IREC 2021-189)

## AUTHOR'S CONTRIBUTION

NAJ and KHAA conceived and designed the study. FHMN and NAJ conducted the literature search, provided research materials, and collected and organized data. FHMN, NAJ, and KHAA analyzed and interpreted the data. FHMN, NAJ, and KHAA wrote the initial and final drafts of the article and provided logistic support. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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# Association between phenotypes and genotype of developmental and epileptic encephalopathy in next-generation sequencing methods in infants: A scoping review

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## ABSTRACT

**Introduction:** Developmental and epileptic encephalopathy (DEE) is epilepsy related to developmental impairment that may be caused by both the underlying etiology (developmental encephalopathy) and superimposed epileptic activity (epileptic encephalopathy). The origin of DEE and the causes of its variations remain unknown. Owing the lack of clarity regarding the role of genetic variables in DEE, we conducted a scoping review to qualitatively identify the genes most important in the development of DEE to provide an up-to-date review.

**Material and methods:** We searched all published studies related to the genetic factors of DEE. The identified publications were screened and selected by the authors on basis of on inclusion and exclusion criteria and assessed for methodological quality. Eighteen articles were included. The extracted data included age of onset, sex, gene mutations and inheritance (e.g. nucleotide change, protein change, and family testing), clinical manifestation, electroencephalogram, imaging, medication, and outcomes.

**Result:** A total of 18 studies were included in this scoping review. The most frequently reported gene variants were STXBP1 in Ohtahara Syndrome, SLC1A2 in Early Myoclonic Encephalopathy (EME), CDKL5 in West Syndrome, SCN1A in Dravet Syndrome, and KCNT1 in Epilepsy of Infancy with Migrating Focal Seizures (EIMFS). Each gene was associated with distinct electroclinical features, including differences in age of onset, seizure type, EEG patterns, and developmental outcomes. While genotype and phenotype associations were heterogeneous, certain variants showed consistent patterns indicative of more severe disease courses.

**Conclusions:** This review identified key gene variants commonly associated with early-onset DEE in infants, particularly STXBP1, SLC1A2, CDKL5, SCN1A, and KCNT1, each linked to unique clinical presentations and outcomes. These findings support the clinical utility of next-generation sequencing (NGS) for early diagnosis and tailored treatment

planning in DEE. Understanding genotype–phenotype correlations may enhance prognostication and highlight potential avenues for targeted therapy in future research.

## KEYWORDS:

*Developmental and epileptic encephalopathy, infant, genetic, scoping review*

## INTRODUCTION

International League Against Epilepsy (ILAE) proposed the term developmental and epileptic encephalopathy (DEE) in “2017 Classification of the Epilepsies” to describe epilepsy related to developmental impairment that may be caused by both the underlying etiology (developmental encephalopathy) and superimposed epileptic activity (epileptic encephalopathy).<sup>1</sup>

Epilepsy incidence generally varies according to age, with younger children and individuals aged 65 years or older experiencing the highest frequencies (>60 per 100,000 individuals). Several population-based studies reported that younger children experience epilepsy at considerably higher rates than older children (82.1–118 vs. 46 per 100,000 person-years). Zuberi et al. reported that neonatal and infant epilepsy syndromes can be broadly categorized into the following two groups: self-limited epilepsies (where there is a decent possibility of spontaneous remission) and DEE.<sup>2</sup> In the newest classification of epilepsy, which is defined by ILAE, syndromes with developmental and/or epileptic encephalopathy or with progressive neurological deterioration were combined, subsequently, the categories were divided by the age of onset.<sup>3–5</sup>

Recent studies have shown that genetic causes have been identified in different epileptic encephalopathies, and various previously unknown genes have appeared.<sup>6</sup> Several of these syndromes are associated with genetic etiology. Development in genomics technology, including next-generation sequencing, has allowed us to discover genes involved in various disorders.<sup>6,7</sup> Genetic testing has proven instrumental

*This article was accepted: 11 June 2025*

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in improving diagnostic yield, informing treatment decisions, and guiding genetic counseling.

Despite these advances, a comprehensive synthesis of the genetic landscape of DEE in infants, especially regarding genotype-phenotype correlations remains lacking. This gap underscores the need for a scoping review that systematically maps current evidence on the genetic etiology of DEE in this age group.

This review aims to answer the following research question: Which gene mutations have been most frequently reported in infants with DEE, and what are the associated clinical phenotypes and inheritance patterns. Accordingly, the objective of this scoping review is to identify and summarize the most commonly reported genetic variants associated with DEE in infants, based on studies utilizing next-generation sequencing technologies.

## MATERIALS AND METHODS

### Protocol registration

This scoping review protocol was not prospectively registered. Although prospective registration is increasingly encouraged to promote transparency and minimize bias, the review was developed and conducted prior to formal registration. Nevertheless, all methodological steps followed established scoping review frameworks to ensure rigor and reproducibility.

### Search strategy

A comprehensive search was conducted across multiple databases, including Cochrane, PubMed, BMC, BMJ, ScienceDirect, EBSCOhost, ProQuest, Google Scholar, and Sage Journals, up to August 2022. Search terms were selected to capture relevant studies on the genotype-phenotype relationships in developmental and epileptic encephalopathies (DEE). These terms were grouped into disease-related and diagnostic method-related categories.

#### a) Disease-related terms included:

"epileptic encephalopathy", "early onset epileptic encephalopathy", "Ohtahara syndrome", "early myoclonic epilepsy", "epilepsy in infancy with migrating focal seizure", "West syndrome", "infantile spasm", "Dravet syndrome".

#### b) Diagnostic method-related terms included:

"genetic testing", "next-generation sequencing", "gene panel", "whole exome sequencing", and "whole genome sequencing".

The terms were combined using Boolean operators (AND/OR) to ensure comprehensive coverage of relevant studies. For example, the search string for PubMed was as follows: ("epileptic encephalopathy" OR "early onset epileptic encephalopathy" OR "Ohtahara syndrome" OR "early myoclonic epilepsy" OR "epilepsy in infancy with migrating focal seizure" OR "West syndrome" OR "infantile spasm" OR "Dravet syndrome") AND ("genetic testing" OR "next-generation sequencing" OR "gene panel" OR "whole exome sequencing" OR "whole genome sequencing").

## Eligibility criteria

Research articles were selected and published between 2012 and 2022. To ensure the validity of the search strategy, all research articles included in our scoping review were assessed with inclusion and exclusion criteria. Observational studies, such as case-control, cross-sectional, and cohort studies, were included in this study. Self-reported surveys, reviews, case reports, case series, editorials, commentaries, and expert opinions were excluded. All participants had to meet the following criteria: age of onset between 0-18 years (pediatric patients), and genetic diagnostic methods used were either whole exome sequencing (WES), whole genome sequencing (WGS), or multiple gene panels performed on more than 50 genes. Moreover, the articles had to specifically identify gene mutation-related DEE. Articles that were not written in English and studies that excluded data taken from human beings were also excluded.

## PRISMA-ScR Checklist

To improve the quality and transparency of scoping reviews, we followed the PRISMA-ScR checklist, which is available in the Supplementary Materials. This checklist was completed and included as Supplementary Material to ensure adherence to reporting standards and to help readers assess the completeness of the review process.

## Data analysis

Mendeley software was used to identify duplicate records and facilitate assist in the screening of titles, abstracts, and full texts based on predefined inclusion and exclusion criteria. The primary reviewer (AT) and additional reviewers (ESH and G) conducted the article screening and selection. Data validation was performed collaboratively by all reviewers, with additional assistance from KHM and KI. Any disagreements were resolved through discussion.

The extracted data included patient characteristics (such as age of onset and sex), genetic findings (including gene mutations, inheritance patterns, nucleotide and protein changes, and results of family testing), clinical features, EEG findings, neuroimaging results, treatments, and outcomes.

As this is a scoping review, a formal quality assessment of included studies was not conducted. The aim was to map the current evidence and highlight key areas for future research, clinical guidance, and policy development,

## RESULTS

### Study characteristics

A total of 8,414 records were identified through database searching. After duplicate records were eliminated, 8,303 records. Next, following title and abstract screening, 7,893 records were eliminated. The eligibility of 410 full-text entries was evaluated in accordance with the inclusion/exclusion criteria. Finally, 18 distinct studies were included in this analysis following the exclusion of 32 full-text articles owing to various reasons indicated in the Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA) flow diagram in Fig. 1

Table 1: Papers included in scoping review

No.	Author	Title	Year	Study type	Sample size	Genetic test
1	Costain, et al. <sup>32</sup>	Clinical Application of Targeted Next-Generation Sequencing Panels and Whole Exome Sequencing in Childhood Epilepsy	2019	Retrospective cohort study	197	WES and TNGS (189 genes)
2	Myers, et al. <sup>33</sup>	De Novo Mutations in SLC1A2 and CACNA1A are Important Causes of Epileptic Encephalopathies	2016	Cohort	531	Targeted sequencing (290 genes)
3	Kothur, et al. <sup>4</sup>	Diagnostic yield of targeted massively parallel sequencing in children with epileptic encephalopathy.	2017	Cohort	141	Targeted panel 71 genes
4	Carvill, et al. <sup>34</sup>	GABRA1 and STXBP1: novel genetic causes of Dravet syndrome.	2014	Cohort	67	WES
5	Komulainen-Ebrahim, et al. <sup>6</sup>	Genetic Aetiologies and Phenotypic Variations of Childhood-Onset Epileptic Encephalopathies and Movement Disorders	2019	Cross sectional	12	WES
6	Wang, et al. <sup>7</sup>	Genetic Variants Identified from Epilepsy of Unknown Etiology in Chinese Children by Targeted Exome Sequencing.	2020	Retrospective cohort study	63	Targeted panel (412 genes)
7	Hino-Fukuyo, et al. <sup>35</sup>	Genomic analysis identifies candidate pathogenic variants in 9 of 18 patients with unexplained West syndrome.	2015	Cohort	18	WES
8	Mitta, et al. <sup>27</sup>	Genotype-phenotype correlates of infantile-onset developmental & epileptic encephalopathy syndromes in South India: A single centre experience.	2020	Retrospective cohort study	82	Multiple gene panel
9	Kobayashi, et al. <sup>36</sup>	High prevalence of genetic alterations in early-onset epileptic encephalopathies associated with infantile movement disorders.	2016	Cohort	11	WES
10	Hamdan, et al. <sup>37</sup>	High Rate of Recurrent De Novo Mutations in Developmental and Epileptic Encephalopathies	2017	Cohort	197	WGS
11	Zhou, P et al. <sup>29</sup>	Novel mutations and phenotypes of epilepsy-associated genes in epileptic encephalopathies.	2017	Cohort	70	Targeted sequencing of 480 genes
12	Peng, et al. <sup>38</sup>	Novel West syndrome candidate genes in a Chinese cohort	2018	Cohort	72	WES
13	Zhang, et al. <sup>39</sup>	Pathogenic variants identified by whole-exome	2020	Cross sectional	43	WES
14	Arafat, et al. <sup>28</sup>	Unexplained Early Infantile Epileptic Encephalopathy in Han Chinese Children: Next-Generation Sequencing and Phenotype Enriching.	2017	Cohort	68	Targeted panel (308 genes)
15	Jiwon Lee, et al. <sup>23</sup>	Determining the best candidates for next-generation sequencing based gene panel for evaluation of early-onset epilepsy	2020	Cross sectional	116	Targeted gene panel on 175 genes
16	Ji-Hoon Na, et al. <sup>26</sup>	Targeted gene panel sequencing in early infantile onset developmental and epileptic encephalopathy	2020	Cross sectional	150	Targeted gene panel on 172 genes
17	Rikke S. Møller, et al. <sup>40</sup>	Gene Panel Testing in Epileptic Encephalopathies and Familial Epilepsies	2016	Cohort	216	Targeted NGS of 46 epilepsy genes
18	Krey, Ilona, et al. <sup>41</sup>	Genotype-phenotype correlation on 45 individuals with West syndrome	2019	Cohort	45	Sequencing panel targeting 131 genes

NGS: Next-generation sequencing; TNGS: Targeted next-generation sequencing; WES: Whole exome sequencing; WGS: Whole genome sequencing;

A total of 18 studies were included in this scoping review (Table 1). Among these, 1 study utilized whole genome sequencing (WGS), 8 applied whole exome sequencing (WES), 13 used targeted gene panels, and 2 employed a combination of WES and panel-based approaches to identify genetic causes of early-onset developmental and epileptic encephalopathies (DEE).

Summary tables of gene mutations associated with distinct DEE syndromes in infants are presented in Tables 2–5. Each table outlines typical genotype and phenotype correlations across specific syndromes, including Early Infantile DEE

(Ohtahara Syndrome and Early Myoclonic Encephalopathy), West Syndrome, Dravet Syndrome, and Epilepsy of Infancy with Migrating Focal Seizures (EIMFS). These summaries highlight patterns in age of onset, seizure types, neurodevelopmental outcomes, EEG features, and treatment response.

Detailed case-level data, including individual nucleotide and protein changes, are provided in the supplementary materials (Supplementary Tables SII–SV).

**Table II: Summary of Gene Mutations Reported in Early Infantile Developmental Epileptic Encephalopathy (EIDEE), including Ohtahara Syndrome and Early Myoclonic Encephalopathy (EME)**

Gene	Age of Onset Types	Common Seizure Clinical	Developmental and Other Features	EEG Pattern	Imaging Findings	Medication and Outcome
<b>Ohtahara Syndrome</b>						
STXBP1	4 days-3 months	Spasms, TS	GDD/ID	BS, HA, MF,	Diffuse atrophy, GSW, GPFA hypomyelination, ventriculomegaly	Often resistant to AEDs
MCAT	~1 day	N/A	DD, dystonia	N/A	BA, cystic necrosis, oedema, thalamic hemorrhage	Drug-resistant
SCN8A	~3 days	TS, GTCS, Myo, FS	GDD	MF, GSW, GPFA, BS	Absent visual regard, spasticity	Partial response to DPH, VPA, CLB
ARX	~53 days	Spasms	Severe ID	BS	Normal	Initial control with ACTH & LEV; later refractory
<b>Others</b>						
KCNQ2 (2 cases). GNAO1, SCN2A						N/A
<b>Early Myoclonic Encephalopathy (EME)</b>						
SLC1A2	~2 d	Myo, TS	Profound ID	MF, slow background	Myelination of cerebellum and brain stem, thin CC, and cerebral atrophy	CBZ, LTG, LCM, pyridoxine, CZP
GABRB2	~7 d	Myo, TS	Severe GDD	BS	normal	AZD, VPA, PB, PHT, prednisolone, VGB, KD, TPM PB, LEV, MDZ, biotin, FOL, B6; intractable seizures

ACTH: Adrenocorticotrophic hormone; AED: Anti-epileptic Drugs; AZD: Acetazolamide; B: Brain Atrophy; BS: Burst-suppression; CC: Corpus Callosum; CLB: Clobazam; CBZ: Carbamazepine; CZP: Clonazepam; DEE: Developmental and Epileptic Encephalopathy; DD: Developmental Delay; DPH: Phenytoin; EEG: Electroencephalogram; FOL : Folic acid; FS: Focal Seizure; GDD : Global Developmental Delay; GPFA : Generalized paroxysmal fast activity; GSW: General Sharp Wave; GTC: General Tonic Clonic Seizure; HA: Hypsarrhythmia; ID: Intellectual Disability; IS: Infantile Spasm; KD: Ketogenic Diet; LCM: Lacosamide; LEV: Levetiracetam; LTG: Lamotrigine; MDZ : Midazolam; MF: Multifocal; Myo: Myoclonic; N/A: Not Available; OS: Ohtahara Syndrome; PB: Phenobarbital; PHT: Phenytoin, TPM: Topiramate; TS: Tonic Seizure; VGB: Vigabatrin; VPA: Valproic Acid

**Table III: Summary of Gene Mutations Reported in West Syndrome/Infantile Spasms**

Gene	No. of Cases	Age of Onset	Common Seizure Types	Developmental and Other Clinical Features	EEG Pattern	Imaging Findings	Medication and Outcome
CDKL5	16 cases	1 day-7 month	IS, TS, GTCS, Myo	GDD, ID, movement disorder	HA, MF	Often normal, CA	Often intractable; Seizure reduction reported with ACTH
STXBP1	8 cases	1 day-9.5 months	IS, Myo, GTCS	ID, GDD	HA, GSpW	Often normal, BA	Often controlled; seizure freedom reported with VPA + TPM
KCNQ2	6 cases	1-4 days	IS, GTS	GDD, dystonia	BS, SpW	Often normal, BA, Thin CC, delayed myelination	N/A
NTRK2	3 cases	3 days-4 months	IS, FS, TS, FIA	Severe GDD, severe ID, microcephaly, hypotonia	HA, MF, DS	Optic nerve hypoplasia	Often intractable; partial response to prednisolone, ACTH, VGB
SCN2A	3 cases	5-10 months	IS, GTCS	GDD, movement disorders	BS, HA	CA	Often controlled; seizure freedom with LTG and CLB
SPTAN1	2 cases	2 weeks-5 months	IS, Myo	ID, acquired microcephaly, hypotonia	Sp, SW, HA, MF	Delayed myelination, CA, thin CC	Often resistant to AEDs
RAB11A	2 cases	4-11 months	IS, Myo, FS, FIA, GTCS	Severe GDD, severe ID, hypotonia	Modified HA	Atrophy, delayed myelination, increased subarachnoid spaces	Seizure reduction reported with LEV and LTG
Others	~20 cases	Variable	IS, TS, GTC, Myo	Variable	Variable	Variable	

ACTH: Adrenocorticotrophic hormone; AED: Anti-epileptic drugs; BA: Brain atrophy; BS: Burst-suppression; CA: Cerebral Atrophy; CC: Corpus callosum; CLB: Clobazam; DS: Diffuse slowing; FIA: Focal impaired awareness; FS: Focal seizure; GDD: Global developmental delay; GSpW: General spike and wave; GTCS: General tonic clonic seizure; GTS: General tonic seizure, HA: Hypsarrhythmia; ID: Intellectual disability; IS: Infantile spasm; LEV: Levetiracetam; LTG: Lamotrigine; MF: Multifocal; Myo: Myoclonic; N/A: Not available; sG: Secondary generalized seizure; Sp: Spike; SpW: Spike and waves; SW: Sharp waves; TPM: Topiramate; TS: Tonic seizure; VGB: Vigabatrin; VPA: Valproic acid

Table IV: Summary of Gene Mutations Reported in Dravet Syndrome

Gene	No. of Cases	Age of Onset	Common Seizure Types	Developmental and Other Clinical Features	EEG Pattern	Imaging Findings	Medication and Outcome
SCN1A	Most reported	Varied between 2 days-11 months	Febrile seizure with FT, GT, FIA, FHC, GAA, GMA, GTC, FTC, GM, FHK, Ab, H, SE, CPS, sGTC	Mild to Severe DD/ID	GSW, MF, PPR, PHR, SW	Often normal, but few BA reported	Often drug-resistant. Partial response reported with combination of OXC, VPA, TPM, CLB, ZNS, LEV, PB, KD
SCN1B	2 cases	~ 6 months	Febrile seizure with Ab, Myo, Febrile SE, GTCS	ID, Regression	Sometimes Normal; PHR SW	Normal	Partial responder to combination of VPA, LEV, CLB, ZNS, PB, and CLZ
GABRA1	4 cases	N/A	Febrile seizure with Ab, At, FDS, H, Myo, TS, GTCS, SE	Mild to Moderate ID	Sometimes normal; GSW, MF, FD, PPR	Often normal	N/A
STXBP1	3 cases	N/A	Febrile seizures with Ab, Ab FDS, Myo, T, TCS, SE	Often Severe ID	MF	Normal; BA	N/A
PCDH19	1 case	~ 4 months	Febrile seizure with Ab, At, FDS, FT, GT	Mild DD/ID	N/A	N/A	N/A
CHD2	1 case	N/A	Absences with head drop, fever provoked FS	Age-appropriate	Occipital, PHR SW, Eye closure sensitivity	Normal	Remission with combination of VPA, CLB, ZNS
CACN1A	1 case	N/A	Febrile seizure with GTC, Myo, CPS	ID	FSW	Normal	Resistant to AED

Ab: absence; AED: Anti-epileptic drugs; At: atonic; CLB: Clobazam; CPS : Complex partial seizure; FDS: Focal dyscognitive seizures; FHC: Focal hemiclonic; FHK: Focal hyperkinetic; FIA: Focal impaired awareness; FSW: Fast spike wave; FT: Focal tonic; FTC: Focal tonic clonic; GAA: Generalized atypical absence; GM: Generalized myoclonic; GMA: Generalized myoclonic absence; GT: General tonic; GSW: General spike wave; GTC/GTCS: General tonic clonic seizure; H: Hemiclonic; ID: Intellectual disability; MF: Multifocal; Myo: Myoclonic; N/A: Not available; PHR: Posterior head region; PPR: Photo-paroxysmal response; SE: Status epilepticus; sGTC: Secondary generalized tonic-clonic seizure; SW: Sharp waves; TS: Tonic seizure; TCS: Tonic clonic seizures; VPA: Valproic acid; ZNS: Zonisamide

Table V: Summary of Gene Mutations Reported in Epilepsy of Infancy with Migrating Focal Seizures (EIMFS)

Gene	No. of Cases	Age of Onset	Common Seizure Types	Developmental and Other Clinical Features	EEG Pattern	Imaging Findings	Medication and Outcome
KCNT1	5 cases	1 day-1 year	FS, TS, Myo, GTCS, CPS	ID	MF, GSW, BS	Often normal; BA	Partial responder to VPA, LEV, TPM
CACNA1A	2 cases	4-5 weeks	FS, TS, GTCS	Severe ID	Migration, MF, GPFA, BA	Normal or delayed myelination	Not responder to LEV, STP, CBZ, VPA, TPM, RFM
SCN2A	2 cases	2-3 days	CPS, GTC	ID	MF	TL atrophy, BA	Controlled to AED
CLCN4	1 case	1 month	CPS	ID	MF	N/A	Remitted to AED
KCNQ2	1 case	2 days	CPS, SPS, GTCS	ID	MF	N/A	Controlled to AED

AED: Anti-epileptic Drugs; B: Brain Atrophy; BS: Burst-suppression; CBZ: Carbamazepine; CPS: Complex partial seizure; EEG: Electroencephalogram; FS: Focal Seizure; GPFA: Generalized paroxysmal fast activity; GSW: General Sharp Wave; GTCS: General Tonic Clonic Seizure; ID: Intellectual Disability; LEV: Levetiracetam; MF: Multifocal; Myo: Myoclonic; N/A: Not Available; RFM : Rufinamide; SPS: Simple partial seizure; STP: Stiripentol; TL: Temporal lobe; TPM: Topiramate; TS: Tonic Seizure; VPA: Valproic Acid

### Early Infantile Developmental Epileptic Encephalopathy (EIDEE)

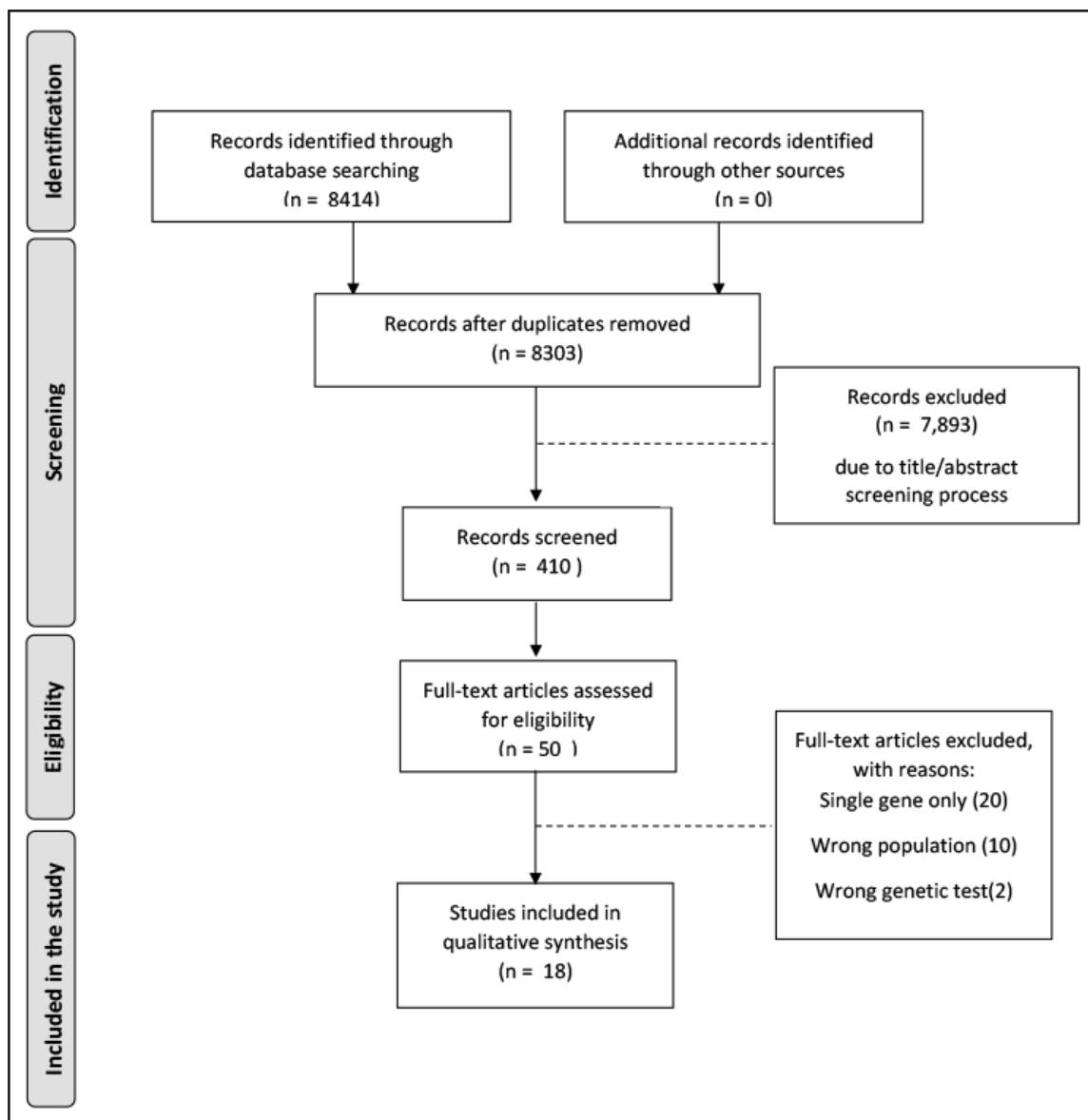
#### Ohtahara syndrome (OS)

Based on the included studies, Ohtahara syndrome was associated with a range of genetic variants, most frequently involving STXBP1 and KCNQ2, followed by MCAT, SCN8A, ARX, GNAO1, and SCN2A (Table II). Reported protein changes included p.Ala294Val, p.Ala306Val, p.Tyr231Cys, p.Arg856Gln, p.Pro480Leu, and p.Ile240Leu, while nucleotide changes were noted at c.592G>A, c.692A>G, c.2567G>A, c.881C>T, c.917C>T, c.1439C>T, and c.718A>C (see Supplementary Table SII). Most variants were de novo, and the age of seizure onset was typically within the first

week of life, with a male predominance. Clinical manifestations included tonic and spasm seizures, with burst suppression as the most common EEG pattern. Treatment responses were generally poor, especially in STXBP1, MCAT, and ARX.

#### Early Myoclonic Encephalopathy (EME)

In EME cases, genetic variants were reported in SLC1A2 and GABRB2 (Table II), with nucleotide and protein changes including c.851C>A and p.Thr284Lys (see Supplementary Table SII). Seizure onset occurred at or before one week of age, and sex distribution was equal. Seizure types were predominantly myoclonic, followed by tonic seizures. EEG



**Fig. 1:** Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA) flow diagram showing systematic literature search and screening

findings showed multifocal discharges, slow background activity, and burst suppression. No family testing results were reported in the included studies.

**West syndrome (WS)**

The most frequently implicated gene in WS or infantile spasm was CDKL5, followed by STXBP1 and KCNQ2, with various nucleotide and protein alterations (Table III). Most reported variants were de novo. Age of seizure onset ranged from 1 day to 11 months, with a female predominance. The most common seizure types were myoclonic and tonic seizures, and EEG frequently showed multifocal discharges, slow background, and burst suppression.

Treatment responses varied: while some patients remained pharmacoresistant, others showed partial improvement with Adrenocorticotrophic hormone (ACTH), vigabatrin, prednisolone, valproic acid (VPA), topiramate (TPM),

levetiracetam (LEV), lamotrigine (LTG), or clobazam (CLB) (see Supplementary Table SIII)

**Dravet syndrome (DS)**

In Dravet Syndrome, genetic variants were found primarily in SCN1A, with diverse nucleotide and protein changes. Additional gene variants identified included SCN1B, GABRA1, STXBP1, PCDH19, CHD2, and CACNA1A (Table IV). Most mutations were reported as de novo based on family testing. The age of seizure onset ranged from 2 to 8 months, with no notable difference between male and female patients. Clinically, febrile seizures were the predominant seizure type. Most patients exhibited severe developmental delay or intellectual disability. EEG findings were variable, and head imaging was typically normal. Regarding seizure outcomes, the majority of patients experienced poor seizure control (see Supplementary Table SIV).

**Epilepsy of infancy with migrating focal seizures (EIMFS)**  
EIMFS cases involved variants primarily in *KCNT1* and *CACNA1A*, with additional reports involving *SCN2A*, *CLCN4*, and *KCNQ2*. Nucleotide changes included c.1038C>G, c.2800G>A, c.1421G>A, and c.4877G>T, resulting in protein changes such as p.Phe346Leu, p.Ala934Thr, p.Arg474His, and p.Arg1626Leu (Table V; see Supplementary Table SV).

Age of onset ranged from 1 day to 1 year, with a higher occurrence in male patients. The most common seizure types were complex partial and generalized tonic-clonic seizures. EEGs often showed multifocal discharges, and brain imaging findings ranged from normal to cerebral atrophy. Most cases showed poor treatment response and severe neurodevelopmental impairment.

## DISCUSSION

Developmental and epileptic encephalopathies (DEE) are progressive disorders characterized by recurrent clinical seizures that contribute to severe developmental delay, regression, and various comorbidities. In 2017, the International League Against Epilepsy (ILAE) proposed a refined classification of DEE, recognizing three overlapping categories: developmental encephalopathy, epileptic encephalopathy, and DEE, where both processes contribute to clinical deterioration.<sup>8-10</sup> The concept of DEE is that in infants presenting with severe early-onset epilepsy, neurodevelopmental comorbidity may be attributable to both the underlying cause and the adverse effects of uncontrolled epileptic activity.<sup>2,8</sup> It appears that if most genes show phenotypic heterogeneity and the most syndromes reveal genetic heterogeneity, the interpretation of this heterogeneity and its significance should be considered in the context of electroclinical presentation.<sup>11</sup>

This scoping review aimed to summarize the gene variants associated with infantile DEE and highlight their electroclinical correlations. In line with prior studies, gene discovery in DEE continues to reveal phenotypic overlap across multiple genes.

Early infantile developmental and epileptic encephalopathy (EIDEE) includes neonates and infants previously classified as having OS and EME. Causative pathogenic gene mutations are commonly found in a majority of patients with EIDEE.<sup>2</sup>

OS was linked to a variety of genetic etiologies, with *STXBP1* being the most frequently reported, followed by *MCA1*, *GNAO1*, *SCN2A*, *SCN8A*, and *ARX*. The most common seizure types were tonic and spasm seizures, and EEGs often demonstrated burst suppression. *STXBP1* is a pathogenic variant noted in 30% of patients with OS.<sup>12</sup> *STXBP1* (more commonly known as *MUNC18-1*) is encoded by the *STXBP1* gene (NM\_003165.3), which comprises 20 exons and is located on chromosome 9q34.11.<sup>13</sup> The *STXBP1* gene encodes the protein known as syntaxin-binding protein, *SEC1/Munc18-1*.<sup>14</sup> It refers to the *SEC1* family of membrane-transport proteins, influence the presynaptic vesicular fusion process by docking and fusing with the synaptic vesicle,<sup>15</sup> and with the haploinsufficiency of *STXBP1* can lead to synaptic vesicle release impairment.<sup>16</sup> The current hypothesis proposes

that the *STXBP1*-E phenotype is caused by loss-of-function mutations in *STXBP1*, which disrupt synaptic vesicular transport and the secretion of neurotransmitters that closely interact with SNARE proteins.<sup>17</sup> In our study, the protein alterations (e.g., p.Ala294Val, p.Ala306Val, p.Tyr231Cys) differed from previously reported common variants such as p.Arg406His, which has been widely associated with *STXBP1*-related epilepsy. A study by Xian et al. grouped 139 individuals (including 49 with OS) into six electroclinical phenotypes and found an association between burst-suppression patterns and p.Arg406Cys/His variants (OR 2.55; 95% CI 1.13–5.46). Similarly, Parrini et al. reported *STXBP1* mutations including p.Arg406Cys in neonatal-onset epilepsy using a 30-gene sequencing panel.<sup>13,14</sup>

In EME, genetic variants were detected in *SLC1A2* and *GABRB2*. *SLC1A2*, located on chromosome 11p13,<sup>18</sup> a key regulator of extracellular glutamate levels. Mutations in this gene lead to impaired glutamate uptake, resulting in excitotoxicity.<sup>3</sup> Clinical manifestations in our study included myoclonic and tonic seizures, profound developmental delay, and EEG patterns with multifocal discharges and burst suppression. Previous reports described a heterozygous missense variant (c.163A>T) in *SLC1A2* associated with generalized epileptiform activity and seizure onset at 40 days of life. Other studies note that *SLC1A2*-related epilepsy often begins with myoclonic or tonic seizures and progresses to multiple seizure types, accompanied by global developmental delay, optic nerve atrophy, and cortical visual impairment.

In the case of *GABRB2*, we observed a variant (c.851C>A; p.Thr284Lys) associated with myoclonic and tonic seizures, severe global developmental delay, and burst suppression on EEG. Previous reports describe different *GABRB2* mutations, such as c.859A>C (p.Thr287Pro) in EME. The *GABRB2* gene encodes a subunit of the GABA-A receptor, a known target of barbiturates, benzodiazepines, and ethanol.

In West Syndrome, the most commonly reported genes were *CDKL5*, *KCNQ2*, and *STXBP1*, with frequent de novo mutations. *CDKL5* encodes a kinase involved in neuronal maturation and gene expression regulation.<sup>19</sup> In our dataset, seizure onset ranged from 27 days to 5 months, with a female predominance.

The most frequent seizure types were myoclonic and tonic seizures, and EEG patterns commonly showed multifocal discharges and burst suppression. The majority of patients had refractory seizures despite treatment, underscoring the need for early diagnosis and tailored therapeutic approaches. This review identified *SCN1A* as the primary gene associated with Dravet Syndrome (DS). *SCN1A*, located on chromosome 2q24.3, encodes the Nav1.1 sodium channel, predominantly expressed in inhibitory interneurons.<sup>20</sup> Loss-of-function mutations lead to neuronal hyperexcitability and epileptogenesis. Most *SCN1A* mutations were reported as de novo and were consistent with haploinsufficiency as the primary disease mechanism.<sup>21</sup> In our review, seizure onset on DS occurred between 2 and 8 months, aligning with known DS onset patterns.<sup>2,22</sup> Clinical manifestations were dominated by febrile seizures, followed by developmental regression and

intellectual disability. EEG findings were variable, and neuroimaging was often normal. Most patients experienced pharmacoresistant seizures.

EIMFS cases in our review primarily involved KCNT1 and CACNA1A, with additional variants in SCN2A, CLCN4, and KCNQ2. KCNT1 is the major gene and is reported in approximately half of patients of EIMFS.<sup>2,23</sup> The KCNT1 gene, which encodes the sodium-activated potassium channel, is the primary gene and is noted in approximately 50% of patients with EIMFS.<sup>2</sup> KCNT1, which encodes a sodium-activated potassium channel, is the most frequently implicated gene in EIMFS. Reported mutations often cluster in the C-terminal region, although some were identified in the pore and transmembrane domains. In our included studies, most patients had seizure onset between 1 and 3 months, often with tonic seizures featuring focal elements. EEG findings were diverse but typically showed multifocal activity, and imaging was often normal or showed brain atrophy. Most patients experienced severe developmental delay and poor seizure control.

In reviewing the genotype and phenotype correlations, several patterns emerged across syndromes. For example, KCNQ2 variants, frequently reported in Ohtahara Syndrome and West Syndrome, were commonly associated with early neonatal onset and burst suppression patterns on EEG, consistent with more severe DEE presentations.<sup>24-26</sup> Similarly, STXBP1 variants, also linked to both Ohtahara and West Syndrome, were associated with intractable seizures, global developmental delay, and characteristic EEG abnormalities, such as burst suppression in Ohtahara and hypsarrhythmia in West Syndrome.<sup>25-29</sup> In cases of EIMFS, KCNT1 mutations were consistently correlated with multifocal EEG discharges, very early onset, and poor response to antiepileptic treatments, reinforcing its role in more severe phenotypes.<sup>26,27,30</sup> Although genotype and phenotype associations were heterogeneous, these patterns suggest that certain gene variants may be predictive of specific electroclinical features and disease severity in DEE.

Genetics has made significant advancements, which have benefited our capacity to diagnose DEE in the context of clinical practice.<sup>31</sup> Genetic variants may be noted in patients with early onset epilepsy at a high frequency as it is highly suspected to have a genetic origin.<sup>26</sup> A high proportion of infants with early-onset epilepsy have an underlying genetic cause, and early identification via NGS (WES, WGS, or targeted panels) can shorten the diagnostic journey. In addition to confirming diagnosis, genetic insights support prognosis estimation, treatment planning, and even clinical trial eligibility. It can contribute to the prediction of disease progression and assist in making informed decisions regarding treatment options.<sup>31</sup>

#### STRENGTHS AND LIMITATIONS OF THE STUDY

This is the first scoping review to describe genetic factors involved in DEE in infants. All studies included in this review were assessed as being of high quality. However, the limitations of this study included the absence of information

on the participants. To conduct a more comprehensive systematic review and even meta-analysis, more observational research on genetic mutations in DEE is required.

#### CONCLUSION

This review identified several key genetic variants, including STXBP1, SLC1A2, CDKL5, SCN1A, and KCNT1, which are commonly found in infants with developmental and epileptic encephalopathies (DEEs), each associated with different clinical features and implications. Our findings reinforce the growing role of next-generation sequencing (NGS) in supporting early and accurate diagnosis, which can help guide more personalized treatment decisions. By summarizing current genetic patterns in infantile-onset DEE, this review contributes to a clearer understanding of the genetic landscape and highlights the value of incorporating genetic testing into routine clinical care. Future studies should focus on evaluating how early genetic diagnosis influences long-term outcomes and how genetic findings can be translated into targeted therapies, especially in resource-limited settings.

#### FUNDING

No funding was sought for this research

#### AUTHORS' CONTRIBUTIONS

AT, ESH, and G made substantial contributions to the conception and design of the work. AT, KHM, and KI contributed to data acquisition. AT, ESH, KI, and G performed the data analyses and the interpretation of the data. AT, ESH, and KHM drafted the text and prepared the figures. AT, ESH, KHM, KI, and G read and approved the final manuscript. All authors approved the present version for publication and are accountable for all aspects related to the study.

#### ACKNOWLEDGEMENTS

We want to express our sincere appreciation to the individuals whose contributions were integral to the completion of this study. Each author played a crucial role in shaping the research design, collecting and investigating data, and drafting and revising the manuscript.

Collectively, the collaborative efforts of all authors have resulted in a comprehensive and rigorous investigation. We extend our gratitude for the dedication, expertise, and collaborative spirit demonstrated by each contributor throughout the research process.

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# Acknowledgement

## July Issue 2025

**The Editorial Board of The Medical Journal of Malaysia gratefully acknowledge the following individuals for reviewing the papers submitted for publication:**

1. Dr Ahneez Abdul Hameed
2. Dr Amanil Ula Hassan
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