Prevalence of Haemoglobin Constant Spring in the Malaysian population: Insights from a single-center study

Sharifatul Fatma Embong, BS[1,2], Adibah Daud, MPath[1,2], Mohammad Hudzaifah Nordin, MMed[2], Sumaiyah Adzahar, MPath[1,2]

[1]Department of Pathology and Medical Laboratory, Hospital Universiti Sultan Zainal Abidin, Terengganu, Malaysia, [2]Faculty of Medicine, Universiti Sultan Zainal Abidin, Terengganu, Malaysia

ABSTRACT

Introduction: Thalassemia is a significant public health concern in Malaysia, with an estimated 6.8% of the population being carriers. These individuals may experience varying severities of anaemia. Alpha thalassemia, in particular, results from deletions or mutations within the α-globin gene complex, leading to reduced or absent α-globin chain production. Haemoglobin Constant Spring (Hb CS) is one of the most common non-deletional alpha-thalassemia variants, characterized by a specific mutation in the HBA2 gene.

Materials and Methods: A cross-sectional study utilized data from 159 samples collected during the National Thalassemia Screening Program for Form 4 students in Terengganu from 2019 till 2022. Haemoglobin analysis was conducted with the capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC) methods. DNA analysis was performed using multiplex PCR and ARMS to detect both deletional and non-deletional α-thalassemia.

Results: Three different types of Hb CS were observed. Among the 159 samples, 137 (86.2%) were heterozygous, 20 (12.6%) were compound heterozygous, and 2 (1.3%) were homozygous for Hb CS.

Conclusion: These findings offer valuable insights for improving genetic counselling practices and public health strategies, especially in regions with a high prevalence of thalassemia.

KEYWORDS:
Thalassemia, haemoglobin constant spring, high-performance liquid chromatography, capillary electrophoresis

INTRODUCTION

Thalassemia is the most common inherited haemoglobin disorder worldwide, with an exceptionally high prevalence in Southeast Asia, including Malaysia.[1,2] It is one of Malaysia's most prevalent inherited blood disorders, where approximately 4.5% of the population are carriers of either beta thalassemia or alpha thalassemia.[3]

Alpha thalassemia results from impaired production of alpha-globin chains, an essential component of haemoglobin. While the deletional forms of alpha thalassemia are well-documented, non-deletional variants such as Haemoglobin Constant Spring (Hb CS) are less recognized but equally significant due to their clinical implications.[4]

Hb CS is caused by a point mutation in the alpha-globin gene (α2), resulting in a termination codon being replaced by a glutamine codon. This leads to an elongated alpha-globin chain that is unstable and inefficiently produced, exacerbating globin chain imbalance.[5,6] Individuals with Hb CS may present with a spectrum of clinical phenotypes, from asymptomatic carriers to severe forms of alpha thalassemia, especially when co-inherited with other alpha-thalassemia mutations.[4]

Several methods for screening thalassemia include capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC). In CE, a peak will appear in Zone 2 for Hb CS, while in HPLC, Hb CS sometimes produces a small peak in the C window at a retention time of 4.90 to 5.30 minutes.[7,8] Screening programs for thalassemia have been set up for all form four students between the ages of 15 and 16 by the Malaysian Government.[9] This screening program aims to determine the thalassemia carrier rate among students in a secondary school and educate these healthy carriers on the risks and options for preventing the birth of children with severe thalassemia syndromes.[10]

The prevalence of Hb CS varies significantly across different populations, reflecting genetic, geographic, and cultural influences. In Malaysia, some communities' multi-ethnic composition and high consanguinity rates contribute to a diverse hemoglobinopathy landscape. However, comprehensive data on Hb CS prevalence remains limited, particularly at a population level. This study aimed to determine the prevalence of Hb CS, a subtype of alpha thalassemia, among form four students in Terengganu, Malaysia. By employing advanced diagnostic techniques such as capillary electrophoresis and molecular analysis, we seek to provide insights into the epidemiology of Hb CS in this region. The findings will enhance the understanding of this condition and inform strategies for genetic counselling, population screening, and public health initiatives.

MATERIALS AND METHODS

This cross-sectional study involved Form Four students aged 15 to 16 who were screened for thalassemia as part of the National Thalassemia Screening Program in Terengganu.

This article was accepted: 28 May 2025
Corresponding Author: Sumaiyah Adzahar
Email: srikandimaya11@gmail.com

Table I: Distribution of types of Hb CS based on genotypes findings (n = 159)

Types and co-inheritance	Number of samples (n)	%
Heterozygous Hb CS	137	86.2
Homozygous Hb CS	2	1.3
Compound heterozygous Hb CS	20	12.6

Table II: Distribution of compound heterozygous Hb CS according to genotypes (n = 20)

Types and co-inheritance	Number of samples (n)	%
Beta thalassemia trait	1	5.0
Hb CS Quang Sze	1	5.0
Het IVS-1	1	5.0
Het with α-3.7	11	55.0
Het with α-4.2	2	10.0
Hb CS Malay	1	5.0
Hb CS with α SEA	1	5.0
Hb CS Adana	1	5.0
Hb CS D Punjab	1	5.0
Total	20	100.0

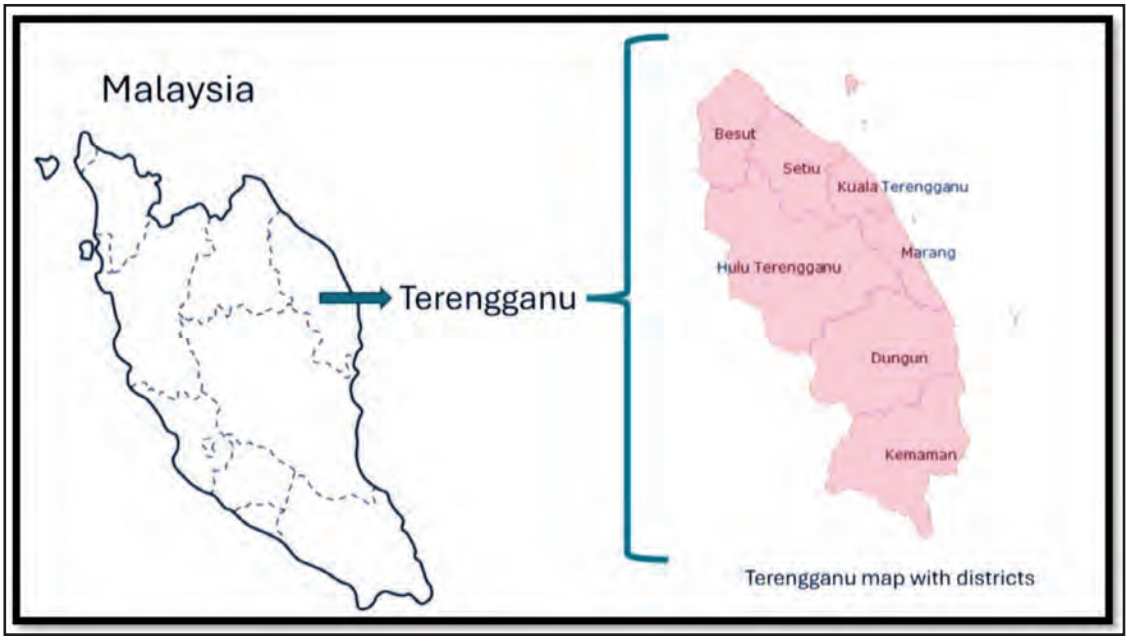

Fig. 1: Map highlighting Terengganu and its districts in Malaysia

Data for the study were collected from the laboratory information system covering the period from January 2019 to December 2022. Access to this data was granted only after obtaining the necessary ethical clearances. Ethical approval for data collection and analysis was secured from the Medical Research and Ethics Committee (NMRR ID-23-00137-ACP) and the Human Research Ethics Committee of Universiti Sultan Zainal Abidin (UHREC) (UniSZA/UHREC/2023/569) prior to the commencement of the study. Additionally, the study adhered to the ethical principles outlined in the Declaration of Helsinki. A total of 159 samples were selected based on a peak at Zone 2 on CE and a small peak at the C window on HPLC. DNA or molecular analysis was conducted using multiplex polymerase chain reaction (PCR) and amplification-refractory mutation system (ARMS) techniques to identify both deletional and non-deletional α-thalassemia mutations. The molecular analysis specifically targeted common α-thalassemia mutations prevalent in Malaysia, including deletional mutations such as $-\alpha^{3.7}$, $-\alpha^{4.2}$, and —SEA, as well as non-deletional mutations such as Haemoglobin Constant Spring (Hb CS; HBA2: c.427T>C) and Hb Adana (HBA2: c.179G>A).

RESULTS

Ethnicity analysis in this study found that 158 (99.4%) of the students with Hb CS were Malays, and 1 (0.6%) was Chinese. No cases were observed among Indian or other ethnic groups. Molecular studies of the 159 samples with a positive peak in Zone 2 on CE confirmed the presence of haemoglobin Constant Spring (Hb CS). Among these, 137 samples (86.2%) were heterozygous for Hb CS, 20 samples (12.6%) were

compound heterozygous, and two samples (1.3%) were homozygous. Of the 159 samples, 54 (34.0%) were from male students, and 105 (66.0%) were from female students (Table I).

The distribution of compound heterozygous Hb CS cases was further analysed and classified based on their genotypes and co-inheritance with alpha or beta-thalassemia. Among these cases, the most common co-inheritance involved Hb CS with the α-3.7 deletion (55.0%) and Hb CS with the α-4.2 deletion (10.0%). One case of Hb CS co-inherited with specific beta-thalassemia variants, including Hb Malay, Hb D Punjab, Hb Adana, and β-thalassemia (Table II).

DISCUSSION

Terengganu, located on the east coast of Peninsular Malaysia, is part of the Southeast Asian thalassemia belt, where hemoglobinopathies are relatively prevalent. It is divided into seven administrative districts with the population predominantly Malay, with smaller Chinese and Indian communities (Figure 1). This ethnic distribution mirrors the demographic composition of Terengganu, where Malays represent the overwhelming majority, followed by minority Chinese and Indian populations.

Terengganu actively participates in the Malaysian National Thalassemia Screening Program, which focuses on identifying early carriers among high-risk groups, such as school students and pregnant women, to prevent disease transmission. Despite these efforts, hemoglobinopathies remain a significant public health challenge due to associated morbidity and the demand for regular transfusions and iron chelation therapy in affected individuals.

Thalassemia traits are highly prevalent in the state, with significant occurrences of both alpha and beta thalassemia carriers. Hb CS, a non-deletional alpha-thalassemia mutation, is one of the common variants in the region. Additionally, other hemoglobinopathies such as Hb E and Hb H disease are frequently reported, often in compound forms, due to co-inheritance of multiple mutations.

This study investigated the prevalence of Hb CS among form four students in Terengganu, Malaysia. The results confirmed Hb CS through molecular studies in all 159 samples collected. Of these, the majority were heterozygous Hb CS, accounting for 86.2%. Additionally, 12.6% were identified as compound heterozygous Hb CS, while 2 samples (1.3%) were homozygous Hb CS. This prevalence aligns with previous studies in Malaysia but highlights the regional genetic burden within the east coast states. The high frequency of Hb CS necessitates targeted public health strategies, including enhanced genetic counselling and community education on hemoglobinopathies.[11]

In cases of compound heterozygous Hb CS, the majority were found to have co-inheritance with the -$\alpha^{3.7}$ deletion. This observation aligns with findings reported by Ramli et al., who also identified the co-inheritance of the -$\alpha^{3.7}$ deletion as the most prevalent form of thalassemia in Malaysia.[12] Among Malaysians, the -$\alpha^{3.7}$ deletion is the most common type of alpha thalassemia, leading to a higher incidence of co-inheritance with Hb CS. Given that Hb CS can lead to clinically significant conditions when co-inherited with other alpha-thalassemia mutations, early detection is essential.[13,14] Public health initiatives, such as the National Thalassemia Screening Program, are instrumental in identifying carriers at an early age, allowing for timely interventions, particularly in high-risk populations. The incorporation of molecular diagnostics into routine screening could further improve the detection of non-deletional alpha-thalassemia variants, ultimately reducing the burden of severe thalassemia syndromes.[15]

This study has several limitations. First, using both capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC) for initial screening may introduce variability in case selection. Second, the sampling was limited to Form Four students from a single Malaysian state, which restricts the generalizability of the findings to the entire country. Additionally, the ethnic distribution was predominantly Malay, reflecting local demographics, but this limits the ability to extrapolate the results to multiethnic populations. Finally, the small number of homozygous Hb CS cases restricts phenotypic analysis for this specific genotype.

CONCLUSION

This study highlights the significant prevalence of heterozygous Hb CS among Form Four students in Terengganu, Malaysia. The co-inheritance of Hb CS with α-thalassemia deletions, particularly the –$\alpha^{3.7}$ deletion, emphasizes the importance of molecular diagnostics in population-based screening. These findings offer valuable insights for enhancing genetic counselling practices and public health strategies in similar demographic areas.

DECLARATION

Authors declare that there is no conflict of interest.

ACKNOWLEDGEMENT

This project was supported by Universiti Sultan Zainal Abidin (UniSZA) under Dana Penyelidikan Universiti (UniSZA/2023/GOT/01).

REFERENCES

1. Bain BJ. (2020). Laboratory techniques for the identification of abnormalities of globin chain synthesis. In Haemoglobinopathy Diagnosis, B.J. Bain (Ed.).
2. Li CK. New trend in the epidemiology of thalassaemia. Best Pract Res Clin Obstet Gynaecol 2017; 39: 16-26.
3. Jameela S, Sabirah SO, Babam J, Phan CL, Visalachy P, Chang KM, et al. Thalassaemia screening among students in a secondary school in Ampang, Malaysia. Med J Malaysia 2011; 66(5): 522-4.
4. Azma RZ, Othman A, Azman N, Alaudin H, Ithnin A, Yusof N, et al. Co-inheritance of compound heterozygous Hb Constant Spring and a single -alpha (3.7) gene deletion with heterozygous deltabeta thalassaemia: a diagnostic challenge. Malays J Pathol 2012; 34(1): 57-62.

5. Pornprasert S, Saoboontan S, Wiengkum T. Hemoglobin Constant Spring (Hb CS) Missed by HPLC in an Hb E Trait Pregnancy Resulting in Hb H-CS Disease in a Thai Girl: Utility of Capillary Electrophoresis. Indian Journal of Hematology and Blood Transfusion 2016; 32(S1): 254-7.
6. Jomoui W, Fucharoen G, Sanchaisuriya K, Nguyen VH, Fucharoen S. Hemoglobin Constant Spring among Southeast Asian Populations: Haplotypic Heterogeneities and Phylogenetic Analysis. PLoS One 2015; 10(12).
7. Greene DN, Pyle AL, Chang JS, Hoke C, Lorey T. Comparison of Sebia Capillarys Flex capillary electrophoresis with the BioRad Variant II high pressure liquid chromatography in the evaluation of hemoglobinopathies. Clinica Chimica Acta 2012; 413(15-16): 1232-8.
8. Singsanan S, Fucharoen G, Savongsy O, Sanchaisuriya K, Fucharoen S. Molecular characterization and origins of Hb Constant Spring and Hb Paksé in Southeast Asian populations. Ann Hematol 2007; 86(9): 665-9.
9. Wee YC, Tan KL, Kuldip K, Tai K, George E, Tan P, et al. Alpha-Thalassaemia in Association with Beta-Thalassaemia Patients in Malaysia: A Study on the Co-Inheritance of Both Disorders. Public Health Genomics 2008; 11(3): 129-34.
10. Tan HY, Hussein N, Lee YK, Abdul Malik TF. Adolescents' experiences and views of the national school-based thalassaemia screening programme in Malaysia: a qualitative study. J Community Genet 2023; 14(4): 361-9.
11. Nahanthiran S, Hafidzah N, Mustapha N, Chellappah Thambiah S, Osman M, Noor SM. Haematologic Profile of Haemoglobin Constant Spring and Its Co-inheritance With Alpha and Beta Thalassaemia Among Form Four Students in Negeri Sembilan. Malaysian Journal of Medicine and Health Sciences 2022; 18(SUPP21): 23-9.
12. Ramli M, Mohd Hassan N, Ramli M, Ab Rahman W, Hassan M, Noor N, et al. Significance of Zone 2 Peak on Capillary Electrophoresis in the Detection of Hemoglobin Constant Spring. Oman Med J 2023; 38(3).
13. Öztürk FN. Alpha Thalassemia in Istanbul: Distribution of Deletions in Alpha-globin Gene Cluster. Genel Tıp Dergisi 2024; 34(3): 376-9.
14. Songdej D, Fucharoen S. Alpha-Thalassemia: Diversity of Clinical Phenotypes and Update on the Treatment. Thalassemia Reports. 2022;12(4):157-172.
15. Wisedpanichkij R, Jindadamrongwech S, Butthep P. Identification of Hb Constant Spring (HBA2: C.427T > C) by an Automated High Performance Liquid Chromatography Method. Hemoglobin 2015; 39(3): 190-5.