A Review of Malaria Research in Malaysia

Timothy William, FRCP* ** *, Jayaram Menon, FRCP** ***

*Infectious Disease Unit, Dept of Medicine, Queen Elizabeth Hospital, Kota Kinabalu, Sabah. **Department of Medicine, Queen Elizabeth Hospital, Kota Kinabalu, Sabah. ***Clinical Research Centre, Queen Elizabeth Hospital Kota Kinabalu, Sabah.

SUMMARY

One hundred and thirteen articles related to Malaria were found in a search through a database dedicated to indexing all original data relevant to medicine published in Malaysia between the years 2000-2013. Thirty eight articles were selected and reviewed on the basis of clinical relevance and future research implications. The epidemiology of malaria has undergone a significant change over the last decade with P. knowlesi, formerly a relatively unknown simian parasite rapidly becoming the most predominant malaria species to infect humans in Malaysia. The epidemiology, clinical features, diagnostic methods and treatment for P. knowlesi infection are described in these studies. In Malaysia, imported malaria from foreigners also poses a challenge. In view of these changes, new strategies on malaria control need to be devised and implemented, and treatment regimens need to be redefined to help Malaysia achieve the goal of malaria elimination by the year 2020.

KEY WORDS: *Malaria, Plasmodium knowlesi, Malaysia, Treatment, Epidemiology*

INTRODUCTION

Malaysia has shown considerable success in controlling malaria. Malaria elimination is now the goal of our country and we aim to be malaria-free by the year 2020. Artemesinin resistance is a challenge to malaria control internationally. However, *Plasmodium knowlesi* cases have increased over the past decade replacing other types of malaria species. It is now the most common cause of malaria in Malaysia, namely in Sabah and Sarawak, and poses a major challenge towards achieving the goal of malaria elimination in our country.

Malaria in humans is caused by five species of *Plasmodium; P. falciparum, P. vivax, P. malariae , P. ovale and P. knowlesi*. The long tailed and pig-tailed macaques (Macaca fascicularis and M. Nemestrina, respectively) are the natural hosts for *P. knowlesi*. These macaques are also the natural host for four other Plasmodium species (*P. cynomolgi, P. fieldi, P. coatneyi and P. inui*)

SECTION 1: REVIEW OF LITERATURE

THE DISCOVERY OF *P. KNOWLESI* MALARIA IN MALAYSIA

The first naturally-acquired case of human knowlesi malaria was acquired in Pahang, a state in the Peninsular Malaysia, in 1965. A second probable case was acquired in Johor a few years later. Knowlesi malaria was thought to be a rare disease until a large focus of human infection was described in Kapit, Sarawak in 2004[1]. Prof Balbir Singh and his team at the Malaria Research Centre at Universiti Malaysia Sarawak (UNIMAS) set out to investigate whether atypical *P. malariae* infections occurring predominantly in adults were attributable to a variant of *P. malariae* or some other Plasmodium species. They discovered using (polymerase chain reaction (PCR) assays, 120 (58%) of 208 patients at Kapit Hospital with malaria tested positive for *P. knowlesi*, whereas none was positive for *P. malariae*. *P. knowlesi* parasites in human erythrocytes were difficult to distinguish from *P malariae* by microscopy. Most of the *P knowlesi* infections were in adults. These infections were successfully treated with chloroquine and primaquine. This report was followed by another major finding by Dr Janet Cox-Singh and the group in UNIMAS, who found that *P. knowlesi* cases were widely distributed throughout Sarawak, Sabah and Pahang, They could also lead to fatal infections[2]. Fread Andreos *et al.* in 2008 and Daw Khin *et al.* in 2011 also described the widespread prevalance of *P. knowlesi* by PCR in Sabah[3,4].

These major scientific discoveries could have enormous implications on malaria control and treatment, mainly for Southeast Asia since every country in this region, except Laos, has described locally-acquired cases of *P. knowlesi*.

EPIDEMIOLOGY

Studies to understand the epidemiology of knowlesi malaria in Kapit by Lee *et al.* of UNIMAS have shown that the prevalence of malaria parasites in wild macaques is very high, with 94% (87/108) of macaques infected[5]. Furthermore, molecular studies on *P. knowlesi* derived from macaques and humans in Kapit, Sarawak have indicated that *P. knowlesi* is an ancient parasite and certain haplotypes are shared between human and macaque hosts. Taken together, these indicate that knowlesi malaria is an ancient zoonosis and humans have been acquiring *P. knowlesi* ever since they ventured into the forests where infected macaques were living. Definitive proof of how long *P. knowlesi* has been infecting humans in Sarawak is not available but a study on archival blood films showed that *P. knowlesi* had in fact already existed in significant numbers throughout Sarawak in 1996[6].

A retrospective review of malaria cases from the Sabah Health Department's malaria notification reports from 1992 to 2011 was conducted by Dr. Timothy William, *et al* to look at the trend of malaria cases in the state over a period of 20 years[7]. Notifications of *P. malariae* and *P. knowlesi* were grouped together. It was found that the total malaria notifications decreased significantly over 20 years. *P. falciparum* notifications peaked at 33,153 in 1994 and decreased 55-fold to 605 in 2011. P. vivax peaked at 15,857 in 1995 and decreased 25-fold to 628

Corresponding Author: tim7008@gmail.com

in 2011. The *P. malariae/P. knowlesi* notifications showed a peak of 614 in 1994 before reducing to less than 100 a year in the late 1990s/early 2000s. The *P. malariae/P. knowlesi* notifications, however, increased 10-fold from 2004 (n = 59) to 2011 (n = 703). In 1992, *P. falciparum, P. vivax and P. malariae/P. knowlesi* monoinfections accounted for 70%, 24% and 1% respectively of malaria notifications, compared to 30%, 31% and 35% in 2011. This showed that despite the decrease in the notification of human malaria, the number of *P. knowlesi* cases had increased significantly in recent years.

In Peninsular Malaysia, malaria is also prevalent but in much lower numbers. Indra *et al* in 2008 discovered that *P. knowlesi* infections also occurred in Peninsular Malaysia. *P. knowlesi* was detected in 77 (69.37%) of the 111 human samples, ten (6.90%) of the 145 monkey blood and in two (1.7%) Anopheles cracens. Sequence of the CSP gene were clustered with other *P. knowlesi* isolates[8].

Ruhani Yusof *et al* also confirmed that *P. knowlesi* was widespread in Peninsular Malaysia[9]. A total of 457 microscopically confirmed, malaria-positive blood samples were collected from 22 state and main district hospitals in Malaysia between September 2012 and December 2013. *P. knowlesi* was identified in 256 (56.5%) samples, followed by 133 (29.4%) cases of *P. vivax*, 49 (10.8%) cases of *P. falciparum*, two (0.4%) cases of *P. ovale* and one (0.2%) case of *P. malariae*. Twelve mixed infections were detected, including *P. knowlesi/P. vivax* (n = 10), *P. knowlesi/P. falciparum* (n = 1), and *P. falciparum/P. vivax* (n = 1). *P. knowlesi* (included mixed infections involving *P. knowlesi* (*P. knowlesi/P. vivax and P. knowlesi /P. falciparum*) showed the highest proportion in Sabah (84/115 cases, prevalence of 73.0%), Sarawak (83/120, 69.2%), Kelantan (42/56, 75.0%), Pahang (24/25, 96.0%), Johor (7/9, 77.8%), and Terengganu (4/5, 80.0%). However *P. knowlesi* infections in Selangor and Negeri Sembilan were found to be 16.2% (18/111 cases) and 50.0% (5/10 cases), respectively. They did not test samples from Kuala Lumpur, Melaka, Perak, Pulau Pinang, and Perlis during the study period and a microscopy positive sample for malaria in Kedah was negative by PCR.

A malaria survey was done in Selangor from 2006 to 201210. The patients were mainly from suburban areas unlike in East Malaysia. A total of 1623 laboratory confirmed malaria cases were reported from Selangor's nine districts; 72.6% of these cases (1178/1623) were attributed to imported malaria, 25.5% (414/1623) were local cases and 1.9% (31/1623) were considered as relapse and unclassified cases combined. In this study, the most prevalent infection was P. vivax (1239 cases, prevalence 76.3%) followed by *P. falciparum* (211, 13.0%), *P. knowlesi* (75, 4.6%), *P. malariae* (71, 4.4%) and *P. ovale* (1, 0.06%). Mixed infections comprising of *P. vivax* and *P. falciparum* were confirmed (26, 1.6%). A case of a patient with imported *P. ovale* infection which was initially misdiagnosed as *P. vivax* was reported.

Seven cases of naturally acquired human *P. knowlesi* infections were admitted to University Malaya Medical Centre in Kuala Lumpur from July 2007 till June 2008[11]. *P. knowlesi* reinfection was also reported in Sabah and in Peninsular Malaysia[12-13]. People may get repeated infections due to a lack of immunity for *P. knowlesi*. Other studies by Gurpreet Kaur *et al* and Norhayati, M *et al* have shown that malaria is common among the Orang Asli people[14-15].

Knowlesi malaria is not the only zoonotic malaria in Malaysia since this year; the first case of naturally acquired human infection of *Plasmodium cyanomolgi*, another malaria parasite of macaques, was reported in Malaysia[16].

THE TRANSMISSION OF *P. KNOWLESI*

Detailed studies on the transmission of knowlesi malaria have been undertaken in Sarawak where Dr Indra Vythilingam of IMR, working in collaboration with researchers at UNIMAS incriminated Anopheles latens as the vector for knowlesi malaria[17]. This vector is found in the forest and forest fringe, feeds predominantly after dusk and is attracted to both macaques and humans[18]. Two other species of mosquitoes (*An. cracens and An. hackeri*) have also been incriminated[8,19].

CLINICAL FEATURES OF *P. KNOWLESI* MALARIA IN ADULTS

A prospective study of the presentation and course of patients with acute *P. knowlesi* infection in Kapit Hospital which is a district hospital in Sarawak from July 2006 to February 2008 was done by Daneshvar C *et al*, from University Malaysia Sarawak (UNIMAS)20. One hundred and fifty two patients were enrolled in the study; 70% had *P. knowlesi*, 16% had *P. falciparum* and 14% had *P. vivax*. *P. knowlesi* infection presented with a non-specific febrile illness and clinical features could not distinguish between knowlesi and the human malarias, *P. vivax* and *P. falciparum*. The base line median parasitemia at admission was 1367 parasites/ml. The knowlesi malaria patients were all thrombocytopenic on admission or the next day. Most (93.5%) of the patients with *P. knowlesi* infection had uncomplicated malaria that responded to chloroquine and primaquine treatment. Seven patients with *P. knowlesi* infection (6.5%) had severe infections at hospital admission. Respiratory distress was the most common complication. Two patients with knowlesi malaria died, representing a case fatality rate of 1.8% (95% confidence interval, 0.2%–6.6%) but larger studies were recommended to determine the case fatality rate for knowlesi malaria.

Another important study was done in Queen Elizabeth Hospital (QEH), Kota Kinabalu, Sabah which is a tertiary hospital by Timothy William, Yeo Tsin Wen and researchers involving more ill patients21. They retrospectively studied patients with *P. knowlesi* malaria diagnosed by PCR from December 2007–November 2009. Fifty-six patients had PCR-confirmed *P. knowlesi* monoinfection and clinical records were available for review. Twenty-two (39%) had severe malaria; of these, six (27%) died. Thirteen (59%) had respiratory distress; 12 (55%), acute renal failure; and 12, shock. None experienced coma. Patients with uncomplicated disease received chloroquine, quinine, or artemether-lumefantrine, and those with severe disease received intravenous quinine or artesunate. Parasite clearance times were 1–2 days shorter with either artemether-lumefantrine or artesunate treatment. *P. knowlesi* was shown to be a major cause of severe and fatal malaria in Sabah.

P. knowlesi malaria in children

In Kudat, Sabah, Barber *et al* studied *P. knowlesi* infection in children[22]. The results showed that *P. knowlesi* in children usually resulted in uncomplicated malaria. They responded well to choloroquine and primaquine. Children commonly had anaemia and knowlesi infection was associated with moderately severe anaemia in addition to thrombocytopenia.

Malaria in dengue endemic areas

In areas that are endemic for dengue, patients presenting with fever and thrombocytopenia are often diagnosed as having dengue fever. Therefore clinicians need to be aware that malaria can also present with similar features. This was highlighted in a retrospective case series done in Peninsular Malaysia by Azira *et al*[23].

COMPARISON OF CLINICAL FEATURES BETWEEN THE DIFFERENT TYPES OF MALARIA SPECIES

A prospective study in QEH by Bridget Barber *et al* from the Queen Elizabeth Hospital (QEH) Infectious Disease Unit and the Menzies School of Health Research, Darwin Australia compared the risk, spectrum, and outcome of severe disease from *P. knowlesi, P. falciparum, and P. vivax* and outcomes following introduction of protocols for early referral and intravenous artesunate for all severe malaria[12]. From September 2010 to October 2011, the researchers prospectively assessed nonpregnant patients aged ≥12 years admitted to Queen Elizabeth Hospital (QEH), Sabah, with PCR–confirmed Plasmodium monoinfection. They found that severe malaria occurred in 38 of 130 (29%) patients with *P. knowlesi*, 13 of 122 (11%) with P. falciparum, and 7 of 43 (16%) with *P. vivax*.

RISK FACTORS FOR SEVERE *P.KNOWLESI* MALARIA

The commonest severity criteria in knowlesi malaria included parasitemia >100 000/μL (n = 18), jaundice (n = 20), respiratory distress (n = 14), hypotension (n = 13), and acute kidney injury (n = 9).

A very important finding was made in this study. On multivariate analysis, *P. knowlesi* was associated with a 2.96-fold (95% confidence interval, 1.19–7.38-fold) greater risk of severity than P. falciparum (P = .020). This clearly shows that P. knowlesi is potentially much more virulent than *P. falciparum*.

Only parasitemia and schizontemia >10% independently predicted knowlesi severity. The risk of severe knowlesi malaria increased 11-fold with parasitemia >20 000/μL, and 28-fold with parasitemia >100 000/μL. Nearly all (92%) knowlesi malaria patients received oral artemisinin therapy; 36 of 38 (95%) and 39 of 92 (42%) with severe and nonsevere disease, respectively, also received ≥1 dose of intravenous artesunate. No deaths occurred from any species.

Another study done earlier by Wilmann *et al* in Sarikei and Sibu, Sarawak showed that patients with high parasite density (≥ 35,000/μl) or with thrombocytopaenia (≤ 45,000/μl) were also more likely to develop complications (odds ratio(OR) = 9.93 and OR = 5.27, respectively)[24].

P. knowlesi is therefore the commonest cause of severe malaria in QEH Kota Kinabalu, with parasitemia the major risk factor for severity. It is recommended that IV artesunate be administered for patients with a parasitemia of >20000/μl for *P. knowlesi*. Early referral and treatment with artesunate was highly effective for severe malaria from all species and associated with zero mortality. This policy should therefore be strictly implemented in Malaysia.

LABORATORY DIAGNOSIS OF MALARIA

Challenges in the microscopic diagnosis of *P. knowlesi*

The only method of diagnosing malaria in hospital laboratories in Malaysia, is by microscopy which has its limitations. Molecular detection methods are more accurate and sensitive but are not rapid, cheap or qualitative so will not replace routine microscopy in rural hospitals where most malaria patients are admitted. Lee, Cox-Singh and Singh studied in detail the morphology of knowlesi malaria parasites They noted that the early trophozoites or ring forms of *P. knowlesi* resembled those of *P. falciparum* and the later erythrocytic stages of *P. knowlesi* were similar to those of *P. malariae*[25]. These findings confirm that it is virtually impossible in routine diagnostic laboratories to accurately differentiate the early ring forms of *P. knowlesi* from those of *P. falciparum*, and the later stages of *P. malariae* with those of *P. knowlesi* by microscopy. *P. knowlesi* trophozoites can also present with an atypical amoeboid morphology as described by a case report by Lee WC et al[26].

In view that Malaysia has five different Plasmodium species that infect humans, a study was done to see how accurate microscopy was to correctly diagnose them. The correct diagnosis is important for treatment and public health surveillance. A prospective study undertaken in QEH Kota Kinabalu Sabah to evaluate the accuracy of routine district and referral hospital-based microscopy by an experienced hospital microscopist, and microscopy performed by an experienced research microscopist, for the diagnosis of PCR-confirmed *P. falciparum*, *P. knowlesi*, and *P. vivax* malaria[27]. Among patients with *P. knowlesi* mono-infection, routine and cross-check microscopy, both identified 94 (72%) patients as "*P. malariae/P. knowlesi*". Routine microscopy identified 17 (13%) as P. falciparum and cross-check microscopy identified 28 (22%). Routine microscopy identified 13 (10%) as *P. vivax* and cross-check microscopy identified two (1.5%). Among patients with PCR-confirmed *P. falciparum*, routine and cross-check microscopy identified 110/122 (90%) and 112/118 (95%) patients respectively as *P. falciparum*, and 8/122 (6.6%) and 5/118 (4.2%) as "*P. malariae/P. knowlesi*". Among those with *P. vivax*, 23/43 (53%) and 34/40 (85%) were correctly diagnosed by routine and cross-check microscopy respectively, while 13/43 (30%) and 3/40 (7.5%) patients were diagnosed as "*P. malariae/P. knowlesi*". Four of 13 patients with PCR-confirmed *P. vivax* and misdiagnosed by routine microscopy as "*P. malariae/P. knowlesi*" were subsequently re-admitted with *P. vivax* malaria. The study concluded that microscopy does not reliably distinguish between *P. falciparum, P. vivax* and *P. knowlesi* in a region like Sabah where all three species occur.

Misdiagnosis of *P. knowlesi* as both *P. vivax* and *P. falciparum*, and vice versa, are common, potentially leading to inappropriate treatment, including chloroquine therapy for *P. falciparum* and a lack of anti-relapse therapy for *P. vivax*.

It is clear that relying solely on microscope diagnosis has its limitations in areas that are endemic for *P. knowlesi*. In this study, it was shown that only 1 out of 117 (0.85%) patients that was reported as *P. malariae / P. knowlesi* by microscopy was confirmed by PCR to actually have *P. malariae*. This is in sharp contrast to the finding that 94 out of these 117 (80.3%) patients was confirmed to have *P. knowlesi* by PCR. This confirms many other important earlier studies that the vast majority of microscopy results in Malaysia which are reported either as *P. malariae* or *P. malariae / P. knowlesi* are in actual fact *P. knowlesi*[1,3,6,11,20,28].

Rapid diagnostic tests (RDTs), while sensitive for the detection of falciparum malaria have not been assessed systematically for knowlesi malaria. A study was done in QEH, Kota Kinabalu, Sabah to prospectively evaluate the sensitivity of two combination RDTs for the diagnosis of uncomplicated and severe malaria from all three potentially fatal Plasmodium species using a pan-Plasmodium lactate dehydrogenase (pLDH)-P. falciparum histidine-rich protein 2 (PfHRP2) RDT (First Response) and a pan-Plasmodium aldolase-PfHRP2 RDT (ParaHIT)[29]. Among 293 hospitalised adults with PCR-confirmed Plasmodium monoinfection, the sensitivity of the pLDH component of the pLDHPfHRP2 RDT was 74% (95/129; 95% confidence interval [CI], 65 to 80%), 91% (110/121; 95% CI, 84 to 95%), and 95% (41/43; 95% CI, 85 to 99%) for PCR-

confirmed *P. knowlesi, P. falciparum*, and *P. vivax* infections, respectively, and 88% (30/34; 95% CI, 73 to 95%), 90% (38/42; 95% CI, 78 to 96%), and 100% (12/12; 95% CI, 76 to 100%) among patients tested before antimalarial treatment was begun. Sensitivity in severe malaria was 95% (36/38; 95% CI, 83 to 99), 100% (13/13; 95% CI, 77 to 100), and 100% (7/7; 95% CI, 65 to 100%), respectively. The aldolase component of the aldolase-PfHRP2 RDT performed poorly in all Plasmodium species. This study showed that the pLDH and the aldolase-based RDT did not demonstrate sufficiently high overall sensitivity for *P. knowlesi*. It was only sensitive for severe cases of malaria with high parasitaemia. Thus the tests may be falsely negative for patients who present with non-severe *P. knowlesi* malaria. Due to its 24-hour replication cycle, this could result in a fatal outcome.

Matthew Grigg *et al* also showed that combining two RDTS showed good specificity but poor sensitivity for the diagnosis of *P. knowlesi* malaria[30].

Foster D et al did a study comparing three RDTS. The RDTs had poor sensitivity and specificity for *P. knowlesi*. Patients with *P. knowlesi* could be misdiagnosed as *P. falciparum* with OptiMAL-IT, *P. vivax* with Paramax-3 and more correctly as non-*P. vivax*/non-*P. falciparum* with BinaxNOW® Malaria31. Therefore, more sensitive RDTs need to be developed for areas that are endemic for *P. knowlesi*.

Paul Divis *et al* reported the analytical and clinical validation of a new real-time PCR assay for *P. knowlesi* based on TagMan technology. The assay showed very good sensitivity, linearity and specificity with plasmid DNA and genomic DNA isolated that was isolated from patients that were infected with *P. knowlesi*. This can be a useful diagnostic tool for *P. knowlesi*[32].

Lau EL *et al* revealed that Loop-mediated isothermal amplification (LAMP) assays could be a potential alternative for molecular diagnosis and routine screening of *P. knowlesi* infection especially in malaria endemic countries, including Malaysia[33]. It could also be useful in monitoring malaria control and eradication programmes.

CLINICAL MANAGEMENT FOR MALARIA IN MALAYSIA

P. knowlesi

Chloroquine in the treatment of uncomplicated *P. knowlesi*

Dansehwar *et al* 's prospective observational study in Kapit, Sarawak showed that oral chloroquine and primaquine was excellent in the treatment of uncomplicated knowlesi malaria, The mean times to 50% (PCT50) and 90% (PCT90) parasite clearance were 3.1 (95% confidence intervals [CI] 2.8-3.4) hours and 10.3 (9.4-11.4) hours. These were more rapid than in a group of 23 patients with vivax malaria (6.3 (5.3-7.8) hours and 20.9 (17.6-25.9) hours; P = 0.02)[34].

Artemisinin Combination Therapy in the treatment of *P. knowlesi* malaria

The clinical studies done in QEH, Kota Kinabalu clearly showed that Artemesinin is effective in the treatment of uncomplicated and severe P.knowlesi. This antimalarial rapidly cleared parasitemia. Therefore policy changes were instituted in the management of malaria in Sabah . All patients with severe malaria were given intravenous artesunate immediately and referred to a Hospital with facilities for Intensive Care.

P. falciparum

The use of Fansidar (Sulphadoxine/Pyrimethamine) in the treatment of *P. falciparum* malaria

Despite the recommendation to use Artemesinin Combination Therapy as first line therapy for the treatment of *P. falciparum* malaria, Fansidar (Sulphadoxine/Pyrimethamine) is still sometimes used in Sabah and Sarawak. Many previous studies have shown that there is a significant resistance to this anti-malarial agent. Sophia Lau *et al* discovered that there was still a high prevalence of mutations in SDX/PYR-associated drug resistant genes in the interior districts of Sabah. This gives further evidence that Fansidar should never be used to treat malaria in Malaysia[35].

DEATHS DUE TO MALARIA

Despite these measures, 14 deaths from malaria were reported in other parts of Sabah during 2010-2011 and studied by Giri Shan *et al* [36].The deaths consisted of seven *P. falciparum*, six *P. knowlesi* and one *P. vivax* (all PCR-confirmed). Of the six *P. knowlesi* deaths, five were attributable to knowlesi malaria and one was attributable to *P. knowlesi*-associated enterobacter sepsis. Patients with directly attributable *P. knowlesi* deaths (N = 5) were older than those with P. falciparum (median age 51 [IQR 50-65] vs 22 [IQR 9-55] years, p = 0.06). Complications in fatal *P. knowlesi* included respiratory distress (N = 5, 100%), hypotension (N = 4, 80%), and renal failure (N = 4, 80%).

It was very notable that all patients with *P. knowlesi* were reported as P. malariae by microscopy. Only two of five patients with severe knowlesi malaria on presentation received immediate parenteral anti-malarial treatment. *P. knowlesi* is much more virulent than *P. malariae* and thus treatment with intravenous artesunate and close monitoring are of vital importance.

The patient with *P. vivax*-associated severe illness did not receive parenteral treatment. In contrast six of seven patients with severe falciparum malaria received immediate parenteral treatment. *P. knowlesi* was responsible, either directly or through gram-negative bacteraemia, for almost half of malaria deaths in Sabah. It was found that patients with severe non-falciparum malaria were less likely to receive immediate parenteral therapy.

The study emphasised the importance for microscopically diagnosed *P. malariae* to be reported as *P. knowlesi* to improve recognition and management of this potentially fatal species. All healthcare workers in the frontlines and clinicians should be informed that they need to treat all severe malaria regardless of the malaria species with immediate intravenous artesunate. Malaria infections including *P. knowlesi*, however, can also present atypically and thus resulting in a delay in diagnosis and management. This can lead to mortality[37].

POST-MORTEM FINDINGS OF P. KNOWLESI MALARIA

Post-mortem findings of a 40-year old male patient who died within two hours of presentation due to severe knowlesi malaria was reported by Cox-Singh *et al* 38. They found multiple petechial haemorrhages in the brain and endocardium. Lungs had features of Acute Respiratory Distress Syndrome (ARDS). Microscopically, there was sequestration of pigmented parasitised red blood cells in the vessels of the cerebrum, cerebellum, heart and kidneys. There was no evidence of any chronic inflammation in the brain or other organs. Brain sections were negative for intracellular adhesion molecule-1. The spleen and liver had abundant pigment containing macrophages and parasitised red blood cells. The

kidney had evidence of acute tubular necrosis and endothelial cells in heart sections were prominent. These findings are similar to fatal falciparum malaria.

SECTION 2: RELEVANCE OF FINDINGS FOR CLINICAL PRACTICE

In view that *P. malariae* and *P. knowlesi* are virtually indistinguishable microscopically and the overwhelming evidence that *P. malariae* is very rare compared to *P. knowlesi* in Malaysia, it is vital to report and notify them as *P. knowlesi* rather than *P. malariae* or *P. malariae / P. knowlesi* (except when the case is imported from a different country). In contrast to *P. knowlesi, P. malariae* which is much more benign rarely causes severe disease. Clinicians also need to be aware that *P. knowlesi* has a higher risk of causing severe malaria compared to the other species and also at lower parasite levels. Early diagnosis and treatment of malaria is very important to reduce mortality. Patients with severe malaria regardless of all species should be treated immediately with intravenous artesunate and closely monitored in a high dependency unit. Both chloroquine and Artemesinin Combination Therapy (ACT) has been shown to be effective for uncomplicated P. knowlesi. The use of an unified blood-stage treatment strategy using ACT for all Plasmodium species should also be considered as correctly diagnosing the malaria species may be challenging.

SECTION 3: FUTURE RESEARCH DIRECTION

There are still a number of gaps in our knowledge in regards to the dynamics of transmission for this infection, including risk factors for transmission, the mosquito vectors, and the occurrence of human-to-human transmission. We also should study the reasons for the changing trend of malaria species in Malaysia. There is also the need for sensitive RDTs capable of detecting knowlesi malaria. We must encourage interdisciplinary collaborative research on malaria among scientific groups from different fields such as entomology, social science, public health, clinical medicine, primatology and others in Malaysia. Research is currently underway in Sabah to define the biomedical, environmental and social risk factors for human infection with Plasmodium knowlesi. This large project named MONKEYBAR is conducted by the Malaysian Ministry of Health in collaboration with the London School of Hygiene and Tropical Medicine, Menzies School of Health Research, Darwin, Australia, University Malaysia Sabah, the Sabah Wildlife Department, University Malaya and other regional partner institutions from the Philippines. At the time of this writing, the Ministry of Health is also collaborating with the Menzies School of Health Research to conduct a randomised control trial comparing ACT with chloroquine in the treatment of *P. knowlesi* (ACTKNOW trial) and in the treatment of *P. Vivax*. These studies are funded by the Malaysian Ministry of Health and the Asia Pacific Malaria Elimination Network (APMEN). A study looking for artemesinin resistance in P.falciparum is also underway.

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