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Determinants of Parasite Clearance Time among *Plasmodium knowlesi* Patients in Malaysia

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Abstract

Plasmodium (P.) knowlesi infection was only recently discovered in 2004, and since then the number of cases has increased each year. As such, there is still a need to understand the clinical management of *P. knowlesi* infection to ensure control of the disease. Therefore, this work was performed to determine factors that affect the clinical outcome of *P. knowlesi* infected patients. A cross-sectional study from 2012 to 2016 was conducted in Hospital Kuala Lipis, Pahang, Malaysia. The main clinical outcome was parasite clearance. A total of 72 *P. knowlesi* patients were included, which were mostly male (n=56, 77.80%), with a mean age of 37.39±14.61 years. Fifty-six patients (77.80%) were prescribed with artemisinin-based antimalarial following diagnosis, while the remaining patients (n=16, 22.20%) were treated with chloroquine. The length for parasite clearance of *P. knowlesi* was 4(4-5) days [(median (IQR)]. Factors that affect the primary outcome of the study (>4 days for parasite clearance) was then determined. A multivariate logistic regression demonstrated that significant predictors of parasite clearance were initial parasite levels and number of symptoms (χ^2=11.97, df(69), P=0.003). Those with an initial parasite count of > 2 400/uL were 4.8 times more likely to take > 4 days for parasite clearance (P=0.02). Having < 4 other symptoms increased the odds of > 4 days for parasite clearance by 0.14 times (P=0.02). This study shows the need to closely monitor patients with higher initial parasite counts (> 2400/uL) and patients with > 4 other symptoms associated with *P. knowlesi* infection, to ensure appropriate treatment is administered.

Keywords: Clinical Outcome, Malaria, Parasite, *Plasmodium knowlesi*

1. Introduction

Malaria is a parasitic infectious disease that affects approximately 3.2 billion people worldwide[1]. Originally known to cause simian malaria, *Plasmodium (P.) knowlesi* is now known as the fifth human malaria species, which is associated with a high risk of severe disease[1]. The emergence of the zoonotic malaria has created an additional challenge in the management of malaria, especially in Southeast Asia[1]. Following its discovery in 2004 in Sarawak, Malaysia, *P. knowlesi* has been the focus of malaria infection among humans[2]. Since then, cases of *P. knowlesi* have been increasingly reported within Southeast Asia, including Indonesia, Thailand, Vietnam, Myanmar and Malaysia[2,3]. *P. knowlesi* infections cause a range of symptoms such as sore throat, chills, rigors, high fever, mild fatigue, anorexia, as well as more severe thrombocytopenia, renal failure, hypotension, jaundice, and deranged liver enzymes that are potentially fatal[1-3]. However, if detected early enough, infections in humans are readily treatable. As a consequence, identifying optimum methods to manage *P. knowlesi* is vital to ensure control of transmission.

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P. knowlesi is a highly pathogenic malaria parasite that can be treated with antimalarials to reduce disease severity[4]. In Malaysia, prior to widespread recognition, *P. knowlesi* was frequently misdiagnosed in *knowlesi*-endemic areas as *P. falciparum* and *P. vivax,* which resulted in delayed management and high fatality rates[5]. With an increase in *P. knowlesi* recognition, this has led to a timelier delivery of pharmacological treatment, reducing case-fatality rates by six-fold[6]. However, due to the limited data on *P. knowlesi* management during the first few years of its emergence[7], older quinolines such as chloroquine were used for management of infection[8,9]. Currently, the use of an artemisinin combination therapy is recommended as the first-line treatment[8,9]. Despite this, deeper understanding of the clinical management of *P. knowlesi* is required for optimal treatment. This is especially an issue due to the steady increase in the incidence of *P. knowlesi* infection within Malaysia[3].

At present, various studies has sought ways to manage *P. knowlesi* through early detection and ways to reduce transmission. If poorly managed, the increase in *P. knowlesi* cases will significantly impact malaria transmission and control. Fortunately, work has shown that the increase of *P. knowlesi* cases could be overcome through various methods such as improving diagnostic capacity, awareness of *P. knowlesi* cases and prompt treatment[3]. Work has also shown that changes in land use patterns increase the opportunity for spill-over of infections to humans, which could be addressed with appropriate monitoring[10]. Careful consideration of treatment is also vital as *P. knowlesi* has a short asexual cycle of 24 hours that can rapidly progress into severe fatal malaria if in appropriately treated[8,9]. Despite these findings, the forecast shows that the increase in *P. knowlesi* incidence rate is expected to continue in the near future[11], demonstrating the need for better understanding in managing *P. knowlesi* infection.

At present, there is limited data that demonstrates factors affecting clinical efficacy of current *P. knowlesi* management. Although there has been great interest in *P. knowlesi* infection, most studies involve risk factors for infection[4,5]. *P. knowlesi* infected patient could present with many complications, and with limited antimalarials available for treatment, identifying predictors of clinical efficacy is vital. Therefore, the aim of the study was to determine the factors that affect clinical outcomes of *P. knowlesi* patients in Malaysia.

2. Materials and Methods

2.1 Ethics

This research was registered with the Malaysian National Medical Research Register (ID NMRR-16-2605-33481) and approved by the Medical Research Ethical Committee of Ministry of Health Malaysia. All procedures performed in studies involving human participants were in accordance to the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

2.2 Study Design

This study was a cross-sectional, retrospective study carried out at Hospital Kuala Lipis, Pahang from January 2012 to December 2016. Kuala Lipis is a largely rural area with a peri-urban environment making it an ideal location to represent both rural and urban areas in Pahang, Peninsular Malaysia. Patients aged 16 years and above admitted to medical wards who were diagnosed with *P. knowlesi* malaria were eligible and enrolled into the study. Patients aged 16 and above are admitted into adult wards in Hospital Kuala Lipis, and thus included into the current study. The exclusion criteria of this study were those who were discharged at their own risk, pregnant or lactating mothers and those with known allergies to any of the antimalarial medications. All patients that fit the study criteria were selected using non-random purposive sampling.

2.3 Data Collection

P. knowlesi was diagnosed using both microscopy and molecular methods of detection as per regular clinical practice[8,9]. Patient characteristics such as demographics (gender, ethnicity, age, nationality), treatment options and clinical characteristics (body temperature, number of symptoms, co-morbidity, initial parasite count, complication, number of days taken to achieve a febrile)[12,13] obtained from the hospital medical record were documented by the researcher on a standard case record form. Fever was a primary symptom of malaria as previously described, while other associated symptoms were categorized as other symptoms.[1,5,7] The standard case record form is used routinely by pharmacist in the Ministry of Health hospitals during daily patient

assessment. Resolution of fever was defined as temperature of less than 37.5°C[3,4]. The primary outcome of the study was the number of days to parasite clearance. Parasite clearance was defined as the time to achieve zero or negative parasite count based on results of Blood Film Malaria Parasite (BFMP) films[3,4].

2.4 Data Analyses

Data were analysed using SPSS (version 23.0) (IBM Corp. Armonk, NY, USA). All continuous variables in the study were found to be not normally distributed and expressed as median and Inter-Quartile Range (IQR). The continuous data included initial parasite count, time taken for fever clearance, number of symptoms and time taken for parasite clearance. Other demographic and clinical data such as age, gender, ethnicity, nationality, occupation, treatment, body temperature on admission, presence of co-morbidities and presence of complications were presented as categorical data. All categorical data were presented in frequency and percentage.

A Mann Whitney U test was performed to compare parasite clearance time between data with two categories. For data with more than two categories, a Kruskal Wallis test was performed to compare parasite clearance time. Non-parametric tests were used as data were not normally distributed.

A univariate and multivariate logistic regression analysis was conducted to identify factors for clinical outcome. Continuous data were categorised based on its median value[14]. The primary clinical outcome was based on the median value, which was >4 days for parasite clearance. All variables with a *P* value <0.05 in the univariate logistic regression analysis were included in the multivariate logistic regression analysis. Both backward elimination and forward selection multivariate logistic regression methods were employed to identify the best predictive model. A confidence interval of 95% was utilised and a *P* value <0.05 was regarded as statistically significant.

3. Results

3.1 Patient Characteristics

A total of 125 patients were diagnosed with *P. knowlesi* over the past five years from 2012 to 2016. There were 5 cases in 2012, 54 cases in 2013, 41 cases in 2014, 18 cases in 2015 and 7 cases in 2016. From an estimated Kuala Lipis population of 100 000, the incidence of the *P. knowlesi* infection was approximately 0.27 cases per 1000 population from 2012 to 2016. Case notes of 53 patients were missing during the study period. The remaining

Table 1. Characteristic of *Plasmodium knowlesi* in the study population from 2012 to 2016 (n=72)

Variables	Value
Gender, n (%)	
Male	56 (77.80)
Female	16 (22.20)
Ethnicity, n (%)	
Malay	36 (50)
Chinese	1 (1.40)
Indian	1 (1.40)
Aboriginal	4 (5.60)
Others	30 (41.60)
Age, years, median (IQR)	32 (26.75-42.5)
Nationality, n (%)	
Malaysian	42 (58.40)
Non-Malaysian	30 (41.60)
Occupation, n (%)	
Working	54 (75.0)
Not-working	18 (25.0)
Treatment, n (%)	
Artemisinin	56 (77.80)
Non-artemisinin	16 (22.20)
Body temperature on admission, n (%)	
Febrile	65 (90.3)
Afebrile	7 (9.7)
Other symptoms, median (IQR)	4 (3-4.3)
Comorbidities, n (%)	
Yes	8 (11.1)
No	64 (88.9)
Initial parasite, (/µL), median (IQR)	2400 (920-9280)
Presence of complication, n (%)	
Yes	16 (22.20)
No	56 (78.80)
Fever clearance, (days), median (IQR)	3 (2-4)

72 cases were included in this study recorded an initial parasite level between 100 - 77 000 /μL (Table 1).

There was a higher number of males (*n*=56, 77.80%), with an overall average age of 37.39±14.60, ranging from 16-77 years old. Foreigners from Bangladesh, Myanmar and Indonesia made up 41.60% (*n*=30) of those infected. It was noted that of those that were working (*n*=54, 75%), most of the subjects (*n*=44, 61.10%) were involved in plantation farms such as rubber tappers, palm oil plantations, and farmers.

Most of the patients presented with the cardinal symptom of malaria which was fever (n=65, 90.3%). However, all patients presented with other symptoms of malaria such as coughing, headache, abdominal pain, chills, sweats, myalgia, nausea and vomiting. Sixteen patients (22.20%) had at least one complication. The common complications observed were acute kidney injury, mild derangement of liver function test and low blood pressure. The co-morbidities (*n*=8, 11.10%) observed were type 2 diabetes mellitus, hypertension and hyperlipidaemia.

Fifty-six patients (77.80%) received artemisinin-based treatment, which were oral artemether 20mg/lumefantrine 120mg twice a day for three days. The remaining 16 (22.20%) patients received non-artemisinin-based treatment, which was oral chloroquine 600mg STAT, followed by 600 mg six hours after the first dose, and then 600mg daily for two days.

3.2 Parasite Clearance Time

Overall, the length of parasite clearance time for *P. knowlesi* patients was 4 (4-5) days [median (IQR)]. Details of patient's characteristics on the primary clinical outcome is presented in (Table 2). Age (*P*=0.02) and initial parasite level (*P*=0.01), were significantly associated with parasite clearance time. Younger patients (*P*=0.02) and those with a lower initial parasite (*P*=0.01) level had a significantly lower parasite clearance time than their counterparts. No other significant findings were observed between patient characteristics and parasite clearance time.

3.3 Determinants of Parasite Clearance Time

A univariate and multivariate logistic regression analysis was performed to identify factors that affect the primary outcome of the study (≥4 days for parasite clearance) (Table 3). Predictors from the univariate analysis with

Table 2. Characteristic of parasite clearance time of *Plasmodium knowlesi* patients (n=72)

Variables	Parasite clearance time (days)	p-value
Gender, median (IQR)		
Male	4 (4-5)	U=414, p=0.62[a]
Female	4 (4-5)	
Ethnicity, median (IQR)		
Malay	4 (3.25-4.75)	H=3.54, p=0.70[b]
Chinese	5 (5-5)	
Indian	5 (5-5)	
Aboriginal	4 (2.5-4.75)	
Others	4 (4-5)	
Age, years, median (IQR)		
<32	4 (4-4)	U=432, p=0.02[a]
>32	4 (4-5)	
Nationality, median (IQR)		
Malaysian	4 (3.75-5)	U=614.5, p=0.85[a]
Non-Malaysian	4 (4-5)	
Occupation, median (IQR)		
Working	4 (4-4.8)	U=437.5, p=0.51[a]
Not-working	4 (4-5)	
Treatment, median (IQR)		
Artemisinin	4 (4-5)	U=545, p=0.12[a]
Non-artemisinin	4 (3.25-4)	
Body temperature, median (IQR)		
Febrile	4 (4-4.5)	U=232.5, p=0.93[a]
Afebrile	4 (4-5)	
Symptoms, median (IQR)[c]		
<4	4 (4-4)	U=660, p=0.56[a]
>4	4 (3-5)	
Comorbidities, median (IQR)		
Yes	4 (4-4.3)	U=277, p=0.69[a]
No	4 (4-5)	

Initial parasite, µL, median (IQR)[c]		
<2400	4 (3-4)	U=435.5, p=0.01[a]
>2400	4 (4-5)	
Presence of complication, median (IQR)		
Yes	4 (4-5)	U=584, p=0.48[a]
No	4 (3.25-4)	
Fever clearance, days, median (IQR)[c]		
<3	4 (4-5)	U=644, p=0.47[a]
>3	4 (3.5-5)	

[a]Mann-Whitney U test: data shows a deviation from normality
[b]Kruskal Wallis test: data shows a deviation from normality
[c]Data were categorised based on median value

a P value <0.05 was then included in the multivariate analysis. The multivariate logistic regression model demonstrated that initial parasite ≥2 400/uL and number of other symptoms ≥4 were independent predictors of having parasite clearance ≥ 4 days when holding all other variables constant (χ^2=11.97, df(69), *P*=0.003). Those with an initial parasite count of ≥2 400/uL were 4.84 times more likely to take ≥4 days for parasite clearance (*P*=0.02). Having < 4 symptoms increased the odds of > 4 days for parasite clearance by 0.14 times compared to their counterparts (*P*=0.02). The model was able to explain 23.9% of the variance in > 4 days of parasite clearance and correctly identified 79.2% of the cases.

4. Discussion

P. knowlesi is a common malaria infection in East Malaysia. However, reports of infection in Peninsular Malaysia are not uncommon, with vectors identified as *Anopheles hackeri* in Selangor and *Anopheles cracens* in Kuala Lipis, Pahang[15]. Both of these mosquito species prefer to feed on monkeys rather than humans[16]. Despite this, the number of *P. knowlesi* reported in Kuala Lipisis slowly increasing. The majority of *P. knowlesi* malaria cases have been reported among adults, with less frequent reports among children. Adults infected are predominanty male possibly due to their active roles in the agricultural field[17]. This likely reflects the environmental interactions that predispose adults that come into contact with infected vectors. In *P. knowlesi* infection, the main worry lies in the fact that high levels of parasitaemia can develop rapidly, potentially causing severe malaria similarly observed in *P.*

Table 3. Clinical outcome predictors of parasite clearance > 4 days for *Plasmodium knowlesi* patients (*n*=72)

Demographics (Ref)	**Beta**	**OR**	**Lower 95% CI**	**Upper 95% CI**	**P-value**
	Univariate Logistic Regression				
Gender (Male)	0.167	1.182	-1.241	1.575	0.816
Ethnicity (Malay)	0.511	1.667	-0.646	1.667	0.387
Age (<32)	0.185	1.203	-0.958	1.327	0.751
Nationality (Malaysian)	0.446	1.563	-0.748	1.640	0.464
Occupation (Working)	0.357	1.429	-1.039	1.752	0.616
Treatment (Artemisinin)	-0.310	0.733	-1.620	0.999	0.643
Fever (Afebrile)	-0.499	0.607	-2.697	1.699	0.656
Symptoms (<4 symptoms)	-1.696	0.183	-3.273	-0.119	0.035
Comorbidities (No)	0.673	1.960	-1.505	2.850	0.545
Initial parasite (<2400/uL)	1.330	3.781	0.071	2.589	0.038
Complications (No)	1.609	5.00	-0.503	3.722	0.135
Fever clearance (<3 days)	-0.922	0.398	-2.295	0.451	0.188
Demographics (Ref)	**Multivariate Logistic Regression**				
Initial parasite (<2400/uL)	1.577	4.840	0.249	2.905	0.02
Other symptoms (<4 symptoms)	-1.942	0.143	-3.578	-0.305	0.02

falciparum malaria[14]. As such, rapid management is vital in patients with *P. knowlesi* infection.

P. knowlesi infection has been treated with a range of antimalarials. In the earlier reports, chloroquine was used frequently for uncomplicated infections, with or without the use of primaquine[13]. However, a comparison of artemisinin-combinations versus chloroquine alone in *P. knowlesi* showed marked efficacy in artemisinin-combinations with lower anaemia rates[18]. The efficacy of artemisinin-combinations has led to its use as a first-line treatment for *P. knowlesi* in the National Antimalarial Guideline[8,19]. This is consistent with the pattern of artemisinin use among the study population in which earlier use of chloroquine was preferred, with subsequent changes to management with artemisinin-combination.

The primary clinical outcome of the study, measured as duration of parasite clearance, was parallel to previous results.[20] P. knowlesi infections reports a relatively shorter parasite clearance time compared to other malaria parasites due to its shorter 24-hour asexual blood-stage cycle[2]. In its natural host, the long-tailed macaque, infection results in prolonged low-level parasitaemia, whereas in rhesus monkeys, parasitaemia rises rapidly and the infection can be lethal[2]. For most patients, parasite clearance occurs within 48 hours[4,20]. The slightly longer parasite clearance time observed in the current work could possibly be attributed to the higher initial parasitaemia level observed in the current setting, which ranged from 100/μL to 77000/μL. This is especially a concern as severity has been shown to be independently predicted by both parasitaemia and schizontaemia levels[4]. The risk of severe P. knowlesi malaria increases 11-fold with parasitaemia levels of more than 20000/μL, and 28-fold with parasitaemia levels of more than 100000/μL.[3,4] In its natural host, the long-tailed macaque, infection results in prolonged low-level parasitaemia, whereas in rhesus monkeys, parasitaemia rises rapidly and the infection can be lethal[2]. For most patients, parasite clearance occurs within 48 hours[4,20]. The slightly longer parasite clearance time observed in the current work could possibly be attributed to the higher initial parasitaemia level observed in the current setting, which ranged from 100/μL to 77000/μL. This is especially a concern as severity has been shown to be independently predicted by both parasitaemia and schizontaemia levels[4]. The risk of severe *P. knowlesi* malaria increases 11-fold with parasitaemia levels of more than 20 000/μL, and 28-fold with parasitaemia levels of more than 100 000/μL[3,4].

Interestingly, the independent predictors of parasite clearance time in the current work were higher initial parasite count (> 2 400/μL) as well as number of other symptoms (< 4). The efficacy of antimalarials are reported to reduce malaria infection with a parasite reduction ratio of approximately 10,000 per erythrocytic cycle for artemisinin, and lower in quinolines[23], demonstrating the effect of higher initial parasite level and longer clearance time. In addition, a higher parasite count has shown to contribute to a higher risk of recurrence[22], supporting the risks of complications associated with a higher initial parasite level. Previous work has also shown that a higher parasite burden significantly delays parasite clearance among children[21], as well as increases the risk of severe malaria[3,4]. Unfortunately, the relationship between parasite clearances is known to be a complex issue, with reported interactions between parasite stage distribution, pattern of drug exposure, as well as parasite killing[20].

Nevertheless, this is the first time the number of other symptoms has been shown to be associated with parasite clearance. The primary symptom of malaria is fever, which is frequently assessed for effectiveness of clinical outcome.[1,5,7] Often, other symptoms observed in malaria are reported to be chills, rigors, lethargy, anorexia, nausea, anaemia, liver enzyme abnormalities, and gastrointestinal disturbances.[2-4] It should also be noted that when the malaria parasites are developing in the liver, no clinical signs and symptoms are usually observed,[22-25] which may suggest a direct association between parasite density and symptoms. The current work demonstrated that in patients with a lower number of other symptoms, parasite clearance time was shorter, which suggests that the number of other symptoms may reflect the level of parasite. However, previous work has shown that other malaria symptoms were not associated with malaria severity and clearance.[24-25] Symptomatic diagnosis of malaria often overestimates prevalence of malaria, while underestimating its severity.[25] Determination of other symptoms apart from fever may also vary between clinicians, with some only noting certain symptoms among malaria patients.[25] The large variation of symptoms between patients may also be due to other underlying diseases and as such, further work needs to be performed to explore these findings.

To that end, we were able to successfully identify predictors of parasite clearance in *P. knowlesi*. Despite the growing interest in *P. knowlesi* since its discovery nearly two decades ago, there is a lack of data looking at predictors

of parasite clearance time in the local setting. Most studies on predicting factors focuses on geographical distribution, spatial distribution and risk of infection[26-28]. As such, the current work suggests the need to closely monitor patients with a higher initial parasite count as well as those with more than four other symptoms, to ensure appropriate diagnosis and rapid treatment is administered. Despite this, we acknowledge that there are a few limitations to the current work. Firstly, being retrospective, there were a few possible confounders. In view of the limited cases in Hospital Kuala Lipis, we collected data of all the malaria patients. In view of the non-random sampling method employed and limited cases present, selection bias could have occurred. Furthermore, being retrospective in nature, other confounding factors such as body weight, social parameters and other medications to name a few were not considered due to the limited information available in medical records that were collected. As such, future work involving a large multi-centered study, taking into account various other clinical factors could address these limitations.

5. Conclusion

The need to control and optimize management of *P. knowlesi* infection is vital and the current work is able to identify ways to address this. The clinical management of *P. knowlesi* can be optimized by ensuring patients with a higher parasite burden and other multiple symptoms are treated rapidly and monitored closely to ensure effective management. There is also a need to further understand the association between other symptoms and parasite clearance time in future work.

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