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PATHOLOGICAL FEATURES OF *Plasmodium knowlesi* MALARIA INFECTION IN HUMANS AND MACAQUES

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REVIEW ARTICLE

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Abstract

Plasmodium knowlesi (*P. knowlesi*), is a simian malaria parasite and currently the dominant species in the Malaysian Borneo of Sabah and Sarawak. This parasite is transmitted by *Anopheles balabacensis* and the macaques of *Macaca fascicularis* and *Macaca nemestrina*, and monkeys of *Presbytis melalophos* are the reservoir hosts. The zoonotic disease, infection by *P. knowlesi* infection can cause a wide range of immunological responses in human comparable to other human malaria parasites including acute respiratory distress syndrome (ARDS), acute renal failure (ARF) and cerebral malaria (CM), leading to the pathological consequences in humans and macaques alike. Similar to other malaria species especially *P. falciparum*, pathological features such as sequestration of parasitised red blood cells (pRBC) and mononuclear cells-containing haemozoin, deposition of haemozoin on numerous tissues and petechial and/or focal haemorrhage could be observed in *P. knowlesi* infection. Diagnosis of *P. knowlesi* mainly involves microscopic examination on both thick and thin blood smears stained with Giemsa, nevertheless confirmation test by using Polymerase Chain Reaction (PCR) is compulsory. Most *P. knowlesi* cases are treated with artemisinin-combination therapy (ACT) or chloroquine in uncomplicated cases while artesunate followed by ACT in complicated or severe cases. Without accurate and timely means of diagnosis and treatment, the outcomes of death might happen. The pathological features of *P. knowlesi* malaria infection in multiple organs are described in this review.

INTRODUCTION

Plasmodium knowlesi (*P. knowlesi*) is a simian malaria parasite that has jumped to be the fifth human malaria parasite [1, 2], an emerging zoonotic disease in Southeast Asia (SEA) [3-6] particularly in the East Malaysia of Sabah and Sarawak [7]. *P. knowlesi* was presumed as a zoonotic infection that has a limited focus on human infections [1, 8]. The misconception was evidenced after the demonstration of a large focus on the parasite infection in the Kapit Divison of Sarawak and the misdiagnosis of *P. knowlesi* infection in human as *P. malariae* [3]. The natural reservoir hosts for the parasite are the long-tailed macaque (*Macaca fascicularis*), pig-tailed macaque *(Macaca nemestrina*) and banded-leaf monkey (*Presbytis melalophos*) [9-13]. Other simian malaria species such as *P. inui*, *P. cynomolgi*, *P. coatneyi* and *P. fieldi* are also commonly found in these natural hosts [10, 11]. The incriminated vectors, definitive hosts for the parasite are the *Anopheles spp.* belonging to the Leucosphyrus group mosquitoes with the following geographical distribution; *A. balabacensis* in the Sabah [14, 15], *A. latens* in Sarawak [13], *A. cracens*, *A. hackeri* and *A.*

introlatus in the Peninsular Malaysia [14, 16-18], and *A. dirus* in Viet Nam [19].

In 2017 alone, there were 2,030 *P. knowlesi* cases reported in Sabah [20]. A large number of *P. knowlesi* cases are arguably contributed by various factors such as deforestation which led to the proximity of humans towards macaques and *Anopheles* mosquitoes [21, 22] and reduction of human malaria cases such as *P. falciparum* and *P. vivax* which may reduce immunity towards *P. knowlesi* infection [23, 24].

Various type of studies has been conducted on utilising *P. knowlesi* as a malaria model either in exploring the host-parasite interactions or drugs and vaccines development [25]. The macaques particularly rhesus macaques (*Macaca mulatta*) often considered as the animal model for the *in vivo* research due to the severity and lethality caused by *P. knowlesi* infections in them [26]. Especially, the histopathological features presented by the macaques yielded a better understanding of the pathophysiology of *P. knowlesi* when compared with similar infections in humans or *P. falciparum* malaria.

BIOLOGICAL FEATURES OF *P. knowlesi*

P. knowlesi possess the shortest asexual, erythrocytic life cycle of 24 hours compared to other human malaria parasites; 48 hours in *P. falciparum* and *P. vivax*, 48-50 hours in *P. ovale* and 72 hours in *P. malariae* [8, 27]. Hyperparasitaemia and the related severe manifestations observed in *P. falciparum* were correlated with the shortest erythrocytic life cycle [7, 28]. *P. knowlesi* has no specific preferences unlike other species and infect both the mature red blood cells (RBC) and the immature reticulocytes [8, 10]. Morphologically, *P. knowlesi* is similar to *P. malariae* in the early trophozoite stage, and *P. falciparum* in both the late trophozoite and schizont stages [29]. Phylogenetically, *P. knowlesi* is closely related to *P. vivax* than other human malaria parasites [30], particularly on the interaction between Duffy binding proteins (DBP) and Duffy antigen receptor for chemokines (DARC) during the RBC invasion phase by the merozoites [31-33]. Molecular analysis revealed that *P. knowlesi* is as old as *P. falciparum* and *P. vivax* [11, 34-37]. Evolutionary analyses of the *P. knowlesi* mitochondrial DNA sequence data indicate that the parasite has existed in the monkeys even before human settlement in Southeast Asia [11].

HISTOPATHOLOGICAL CHANGES DURING *P. knowlesi* MALARIA

The histopathological examinations of *P. knowlesi* malaria revealed the major organ damages [38-41], primarily on the brain, liver, lung, spleen, kidney, and heart that were overlooked (Table 1) similar to *P. falciparum* malaria [44]. The histopathological features from the post-mortem observation of a fatal *P. knowlesi* malaria were comparable with the *P. falciparum* malaria that strengthen the possibility of the similar pathophysiological process [38]**.** There were limited number of studies were reported about the histopathological aspects of *P. knowlesi*. This review will discuss the findings of the histopathological changes observed in humans and macaques.

HISTOPATHOLOGICAL CHANGES IN THE BRAIN

The common features of cerebral malaria (CM) were elaborated in a post-mortem case report [38]. The gross examination of the brain from a fatal human *P. knowlesi* malaria case was noticed with the petechial haemorrhage on the cerebrum and cerebellum, while the brain stem and spinal cord appeared normal [38]. The surface of the cerebrum was also appeared to be dusky. Microscopic observation revealed numbers of prominent sequestration and accumulation of parasitised red blood cells (pRBC) in the microvasculature and large vessels respectively. Petechial haemorrhage was largely derived from the microvasculature of the cerebrum and cerebellum, and the haemorrhagic areas were demonstrated with numerous haemozoin deposition. Intriguingly, there was no evidence of perivascular inflammatory reactions, polymorph nuclear cells aggregation and intravascular thrombosis, as well as negative for the Intracellular Adhesion Molecule-1 (ICAM-1) through immunohistochemistry staining. Generalised encephalitis was not observed as well. Furthermore, the presence of perivascular or diffuse parenchymal oedema, diffuse astrocytosis or microgliosis, and acute gliotic reactions resulting in haemorrhages were not observed. The minimal invasion of acute inflammatory cells and related signs of inflammation including oedema despite the local haemorrhagic events need further evaluation yet can be correlated with the shortest life cycle of the parasite. Also, the cerebral pathology is identical to the fatal *P. falciparum* malaria [38]. Remarkably, despite the presence of focal neurological signs and CM-associated syndrome, the coma or syncope was absent in *P. knowlesi* unlike fatal *P. falciparum* CM cases [28, 29, 38, 47]. Intriguingly, several cases pointed out rare events of coma presented in the severe *P. knowlesi* malaria cases that raise the concern to rule out co-infection with *P. falciparum* [48].

The microscopic observation of the brain tissues from the autopsied rhesus macaques (*Macaca mulatta*) revealed the typical intravascular haemozoin and fibrin deposition [40, 41] as well as petechial haemorrhage at the cortex, demyelinated alba of the cerebrum [40]. Additionally, the observations included the cellular atrophy of the cortical neurons and congested capillaries with sequestered pRBC and RBC-containing haemozoin in the lumen. While in the cerebellum, the presence of malaria parasites and haemozoin deposition in the microvasculature of the cortex and a reduction in numbers of Purkinje cells and granular layer cells were also reported.

Table 1. Histopathological Findings Associated with *P. knowlesi* Malaria

Organs		Features	No. of Cases/Samples	References
Brain	i)	Sequestration/accumulation of pRBC, haemozoin-laden RBC – micro and large vessels	1 (Human), 6 (*M. Mulatta*).	[38, 40]
	ii)	Petechial haemorrhages, haemozoin deposition – cerebrum and cerebellum, cortex		
	iii)	Intravascular malaria parasites, haemozoin and fibrin deposition	18 (*M. Mulatta*).	[40, 41]
	iv)	Demyelinated alba – cerebrum	6 (*M. Mulatta*).	[40]
	v)	Pyknotic cortical nerve cells		
	vi)	Hypoplastic Purkinje and granular layer cells		
Heart	i)	Sequestration of pRBC	1 (Human), 6 (*M. Mulatta*).	[38, 40]
	ii)	Petechial haemorrhage – endocardium	1 (Human)	[38]
	iii)	Focal petechial haemorrhage - subendocardium		
	iv)	Subendocardial haemorrhage – left ventricular wall		
	v)	Intravascular haemozoin and fibrin deposits	12 (*M. Mulatta*).	[37]
	vi)	Fibrin deposits adhered to the vessels, microvessels and endocardium		
	vii)	Atrophic and myolysis of cardiac myocytes		[40]
Lungs	i)	Plugs (pRBC, haemozoin-laden macrophages, mononuclear cells) – pulmonary vessels	18 (*M. Mulatta*).	[40, 41]
	ii)	Microembolism – alveolar capillaries		
	iii)	Pulmonary capillary congestion	12 (*M. Mulatta*).	[41]
Liver	i)	pRBC and haemozoin deposition – Kupffer cells and sinusoids	1 (Human), 6 (*M. Mulatta*), 4 (*M. fascicularis*)	[40, 41, 45]
	ii)	Haemophagocytosis		
	iii)	Moderate-chronic lymphoplasmacytic inflammation – portal tracts and sinusoids	1 (Human)	[38]
	iv)	Severe macrovascular steatosis – hepatocytes		
	v)	REC changes	6 (*M. Mulatta*), 4 (*M. fascicularis*)	[40, 45]
	vi)	Hepatocytes - hydropic, atrophic, necrotic and vacuolar degenerated		
	vii)	Dissociated centrilobular	4 (*M. fascicularis*)	[45]
	viii)	Centrilobular hepatic necrosis	12 (*M. Mulatta*)	[41]
	ix)	Mild lipidosis - hepatocytes		
	x)	Proliferated interlobular fibre	6 (*M. Mulatta*)	[40]
	xi)	Stripping of sinusoidal endothelial cells		
Spleen	i)	pRBC, haemozoin-laden macrophages and haemophagocytosis – red pulp	1 (Human), 18 (*M. Mulatta*), 4 (*M. fascicularis*)	[38-41, 45]
	ii)	Increase in size, dissociated or loss of the germinal centres, congested and expansion of the red pulps, and atrophied and hyperplastic of the white pulps.		
	iii)	Splenic haemorrhages	1 (Human)	[46]
	iv)	REC changes/ and containing haemozoin-laden macrophages – splenic cords.	12 (*M. Mulatta*), 1 (Human)	[41, 46]
	v)	Indistinct marginal zones	6 (*M. Mulatta*), 4 (*M. fascicularis*)	[40, 45]
	vi)	Shrinkage/ contracted of splenic corpuscle	6 (*M. Mulatta*), 4 (*M. fascicularis*)	[40, 45]
	vii)	Haemozoin deposition – whole spleen	2 (Human), 4 (*M. fascicularis*)	[38, 45, 46]
	viii)	Necrotic lymphocytes – germinal centres	12 (*M. Mulatta*)	[37]
	ix)	Distended sinusoid with RBC		
	x)	Hyperplastic lymphoid, surrounded by fibrin		

Kidneys	i)	Sequestration of pRBC	1 (Human)	[38]
	ii)	Intratubular cast formation		
	iii)	pRBC with/without haemozoin – capillaries	6 (*M. Mulatta*)	[40]
	iv)	Thickening membrane of capillaries		
	v)	Contracted renal corpuscle		
	vi)	Vacuolation of tubules	12 (*M. Mulatta*)	[41]
	vii)	Tubular epithelium atrophy and degeneration (hyaline droplet degeneration-renal tubular necrosis)		
	viii)	Pyknotic nuclei and vacuolated cytoplasm	18 (*M. Mulatta*)	[40, 41]
	ix)	Haemoglobin, haemozoin, RBC, granular, protein and cellular casts – collecting tubules		

HISTOPATHOLOGICAL CHANGES IN THE HEART

Cardiac involvement of *P. knowlesi* malaria in humans was reported with haemorrhages at the apex of the heart on gross examination in a fatal case [38]. Microscopically, petechial haemorrhages on the endocardium and subendocardium were observed. The focal petechial haemorrhages on the subendocardium were due to the localised leakage of blood from the ruptured capillaries to the surrounding tissue, might be related to either resuscitation measures; where the patient had collapsed and intubated with adrenalin/atropine and sodium bicarbonate therapy; or secondary to the causes apart from the malaria infection. There were several extensive subendocardial haemorrhages observed on the left ventricular wall, although myofibres of the left ventricle were appeared to be normal. Interestingly, there was no evidence of myocarditis. pRBC sequestrations were observed in the cardiac vessels particularly in the microvessels without the appearance of the chronic inflammatory reactions but the endothelial cells were appeared to be prominent. Additionally, the auscultation of the heart (listening to the heart sounds) was normal before the autopsy was performed. Normal heart sounds are produced in response to the closure of the heart valves, where the first heart sound of S_1 is produced following the closure of the mitral and tricuspid valves (systole) while the second heart sound of S_2 when the aortic and pulmonary valves close (diastole), [39] thus producing the “lubb” in S_1 and “dubb” in S_2.

In the macaques of *M. mulatta*, the overall heart appeared to be “pale tan” and firmly contracted [41]. The peritoneum and mesenteric abdominal fat were yellowish tan. Most of the macaques' heart chambers were evidenced with grey-greenish mural thrombus plaques that adhered to the endocardium. Microscopically, pRBC (Figure 1) was observed in the capillaries [40] and numerous loosely arranged haemozoin and intravascular fibrin deposits containing cells were observed in the vessels [41]. These fibrin deposits adhered to the microvessels, propagated from the endothelium of the cardiac muscles which are suggestive of the disseminated intravascular coagulation (DIC) that were evidenced in the simian malaria infections. Atrophy (reduction in the cell size) and myolysis (destruction of muscle tissue) of the cardiac muscles were also observed [40]. Atrophic of cardiac muscles is associated with congestive heart failure (CHF) and possibly due to factors during malaria infection such as excessive pro-inflammatory cytokines production and multiorgan failures including the liver and kidney [42]. The cardiac myolysis (destruction of the heart muscles) and rhabdomyolysis (destruction of the skeletal muscles) were caused by the obstruction of microvascular by the sequestrated pRBC, which lead to tissue hypoxia [43].

Cardiac complications due to malaria are mostly left unnoticed and precursor, although not often, to hypotension, shock, circulatory collapse accompanied by impaired haemodynamic function and pulmonary complications in certain cases [44]. DIC occurred due to the localised inflammatory responses or cytokine storm, or even circulatory obstruction following the sequestration of pRBC and inflammatory cells, which leads to vasoconstriction and ended up to organ damage due to the impairment of blood transports [41, 49]. Regardless, this event is intricate to be exemplified and probably to be formed before death itself [41, 49, 50] rather than due to the disease manifested by the parasite. Similar to *P. falciparum* malaria, the risk of severe *P. knowlesi* malaria may also be proportionately associated with the underlying cardiovascular-metabolic disease [51].

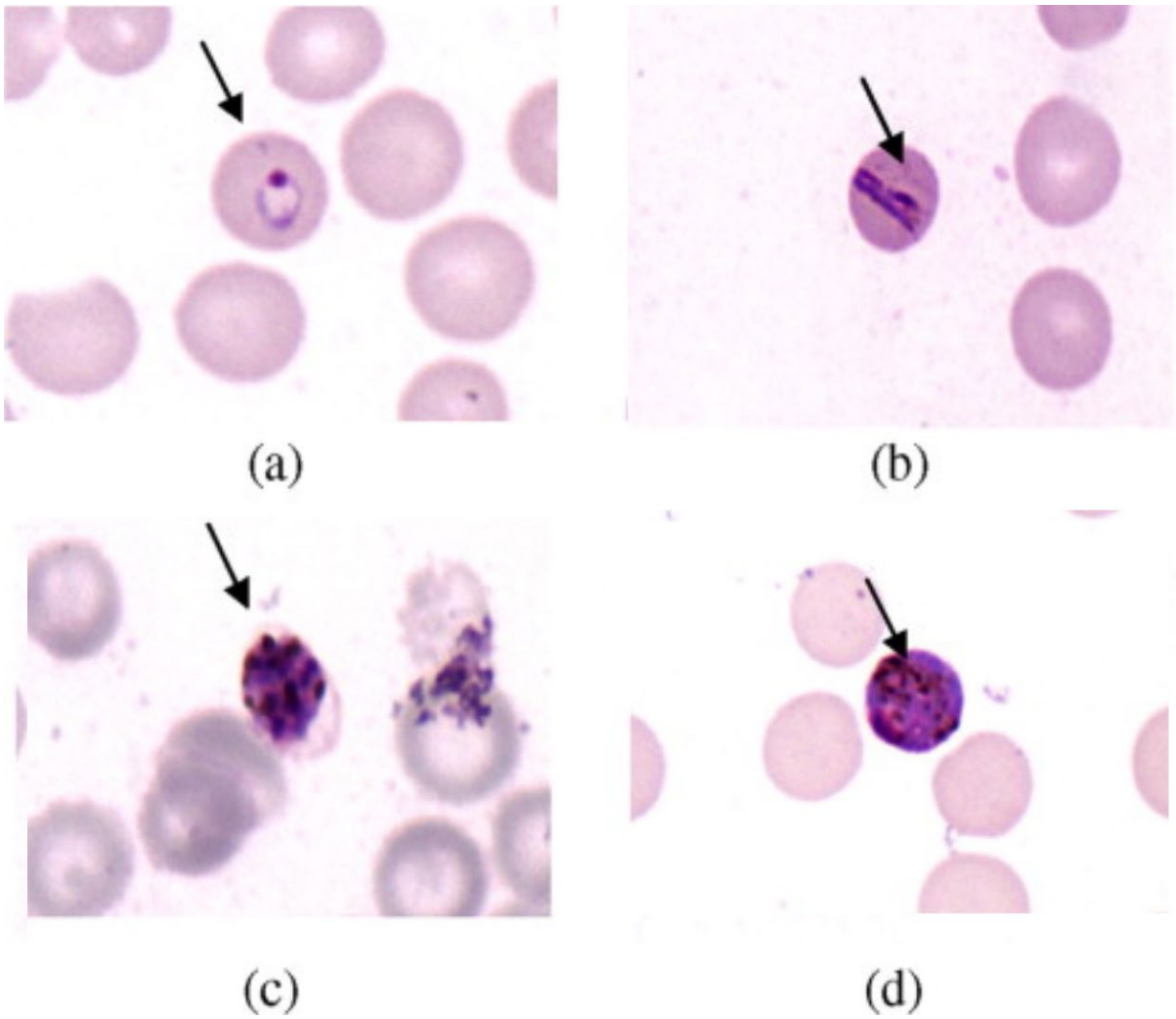

Figure 1. Morphology of *P. knowlesi* in Giemsa-stained thin blood films from the infected macaques. pRBC (black arrow) in form of (a) early trophozoite, (b) late trophozoite form, (c) schizont and (d) macrogamete compared to surrounding, uninfected RBC [45]

HISTOPATHOLOGICAL CHANGES IN THE LUNG

In a fatal human *P. knowlesi* case, the lungs were heavy and the cut sections demonstrated congested and "beefy" (dark-red appearance, these features were correlative with the acute respiratory distress syndrome (ARDS) [38]. Gross specimen examination of the autopsied lung of the *P. knowlesi*-infected *M. mulatta* revealed soft, dry, collapsed lungs with grey-tan in appearance [41]. Some of the macaques had the lungs inflicted with mild pulmonary oedema. Microscopically, the pulmonary vessels were occluded with solid plugs comprised of numerous mononuclear cells [41], as well as pRBC and haemozoin laden macrophages [40] where microembolism was observed in the capillaries of the alveolar. These lungs presented with pulmonary capillary congestion that led to pulmonary oedema [41].

Acute respiratory distress syndrome was the most common complication in knowlesi malaria, associated with metabolic acidosis and pulmonary oedema. Clinical presentation of hypoxemia, tachypnea and pulmonary infiltrates or oedema were associated with ARDS, which were commonly observed in the severe *P. knowlesi* malaria [29]. It is evident that *P. knowlesi* malaria manifests histopathological changes of the lung comparable to other fatal human malaria cases.

HISTOPATHOLOGICAL CHANGES IN THE LIVER

Gross examination of the liver specimens from a fatal *P. knowlesi* malaria in humans demonstrated enlarged (or hepatomegaly) [38]. Under microscopic examination, the liver was evidenced with the reticuloendothelial cells (REC) changes and severe macrovascular steatosis of the

hepatocytes without the evidence of parenchymal inflammation. There was no evidence of vasculitis or perivascular chronic inflammation, regional necrosis or thrombotic microangiopathy. Interestingly, numerous pRBC and haemozoin deposition in Kupffer cells and haemophagocytosis were observed. Additionally, moderate chronic lymphoplasmacytic inflammation in the portal tracts and sinusoids were noticed [38]. In *M. fascicularis*, the macroscopic evaluation revealed firm and hyperaemic, hepatomegalic liver with black in appearance [45]. There were distinct lobes with round edges with the distended (swollen) gallbladder, suggested hepatomegaly (swollen liver) and gallbladder inflammation (cholecystitis), although the underlying mechanisms were largely unknown in malaria [52]. Plausible mechanisms are as follows: ischaemia caused by severe hypotension and haemolytic anaemia; imbalance of pro-and anti-inflammatory cytokines which injured the gallbladder, and the intravascular sequestration by malaria parasites on the vessel walls especially in *P. falciparum* infections which leads to gallbladder ischemia. Microscopic observation demonstrated the dissociation of the centrilobular arrangement with hydropic and necrotic hepatocytes [45]. Atrophy and vacuolar degeneration of the hepatocytes were also observed. It was evidenced that there were hypertrophic, haemozoin-laden and phagocytosed-parasite of Kupffer cells and haemozoin deposition in the sinus throughout the endothelial cells.

Macroscopical gross examination of the livers of infected *M. mulatta*, appeared normal in both size and shape exhibiting grey to brown colour with distinct lobular pattern [41]. Microscopically, phagocytosed malarial parasite, haemozoin-laden Kupffer's cells [40], and hyperplastic of haemozoin-laden Kupffer's cells with centrilobular (zonal) hepatic necrosis was observed in most of the livers [41]. The severity of hepatic necrosis was ranging from the central necrotic zone confluence to the necrosis of the individual hepatocytes surrounding the central veins. There was also vacuolar degeneration, atrophied hepatocytes stripping from the sinusoidal endothelial cells and proliferation of the interlobular fibrous tissues [40]. A diffuse and mild lipidosis was characterised through one or two large lipid droplets in most of the hepatocytes [41]. Additionally, one of the livers had bile stasis due to the endothelial blockage by the pRBC, which disrupted the vascular flow thus resulting in cholestasis, impeding the flow of bilirubin transportation and bile excretion, and hepatic dysfunction [53].

The dark-coloured liver was due to the deposition of the haemozoin, similarly in *P. falciparum* malaria [54]. Histopathological changes in the macaques of *M. fascicularis* [45] and *M. mulatta* [40] are comparatively similar, which may reflect the severity of the parasite towards different species of the macaques as well. Liver functions tests demonstrated an increase in many of the markers in *M. fascicularis*, especially the total bilirubin level was exceedingly high; a precursor to the degree of haemolytic reactions in malaria-infected hosts. Interestingly, in the CM cases, the mean bilirubin levels were four times higher than the upper limit compared to uncomplicated malaria and two times higher when compared to normal levels in humans [55].

HISTOPATHOLOGICAL CHANGES IN THE SPLEEN

In a fatal, human *P. knowlesi* case, the spleen was enlarged (or splenomegaly) and appeared to be soft and viable under gross examination [44]. In a study by Chang et al, (2018) from a total splenectomy patient infected with *P. knowlesi*, the spleen was average in size (12 cm) [46]. The tearing of the splenic capsule at both the superior pole and visceral surface were also observed, where a subcapsular haematoma was evident at the superior pole. There was no visceral perforation. Microscopically, numerous pRBC and haemozoin-laden macrophages were observed, particularly at the red pulp which phagocytosed pRBC, and deposition of the haemozoin pigment were also prominent [38]. Haemozoin deposition was observed throughout the spleen, with REC changes and splenic haemorrhage indicative of the focal breaching of the capsule and the area of haemorrhage, thus suggestive of splenic rupture [46]. The red pulp was congested while the white pulp was atrophied, yet the germinal centres were not observed [38]. Despite this, chronic inflammatory reactions, necrosis and fibrin clot are absent. The spleen of *P. knowlesi*-infected *M. fascicularis* was friable and haemorrhagic on gross examination [45]. The microscopic observation was evidenced with reactive areas with an increase in size and disappearance of the germinal centres with mantle zones and pronounced haemozoin deposition. The congested red pulp demonstrated the presence of pRBC and haemozoin-laden macrophages, while the size of the white pulp was increased with pronounced shrinkage of splenic corpuscles. Additionally, the marginal zone was indistinct.

In *M. mulatta*, infection by *P. knowlesi* evidenced with the macroscopic features of splenomegaly and the spleen was appeared to be firm, dark red-purple while coarse granular areas were observed at the cut surface due to the lymphoid aggregates [41]. Microscopically, the red pulp was congested with the presence of pRBC and haemozoin-laden macrophages [40] and expanded that was more obvious than the white pulp [35]. The splenic corpuscle was markedly contracted [40] while the germinal centres were occupied with the necrotic lymphocytes [41] and dissociated [40]. The lymphoid areas were hyperplastic with most of the lymphoid follicles were presumably surrounded by fibrin and containing the germinal centres [41], while the marginal zone was indistinct with globulin disposition [40]. The sinusoids were distended with RBC, with numerous REC containing haemozoin-laden macrophages on the lining of the splenic cords [41].

The splenic rupture was usually accompanied by internal haemorrhage and highly associated with severe malaria

infection [56]. Splenectomy was usually performed due to such complications [57]. Despite this, splenectomised patients were indicated with haemodynamic instability, yet proper treatment could stabilise and reduce the haemorrhage [46, 58, 59]. Splenic rupture is reported to occur in the cases of low parasitaemia levels [46, 60, 61].

HISTOPATHOLOGICAL CHANGES IN THE KIDNEY

Although gross examination of kidneys was appeared to be normal, post-mortem observation in a fatal human *P. knowlesi* case presented with the features of pRBC sequestration in the vessels and intratubular casts formation, despite the absence of the perivascular inflammatory cells which are commonly expected with chronic inflammatory reactions [38]. These features are indicated with acute tubular necrosis (ATN) and renal failure [7, 38].

In *P. knowlesi*-infected *M. mulatta*, grossly the renal cortex of the kidneys from most of the macaques had punctuated red-brown stippling which extended as numerous fine and linear of interrupted streaks [41]. Haemoglobinuria was also observed in those macaques. Some of the kidneys from other macaques were observed with dark red-brown colour on the cortex and medulla without a prominent difference in colour. As of the microscopic analysis, haemoglobin casts and tubular degenerations were evident, ranging from hyaline droplet degeneration to renal tubular necrosis [41] while vacuolation of the tubules with the presence of pRBC and RBC-containing haemozoin in the capillaries were also indicated [40]. The renal corpuscle was contracted yet the membrane of the capillary was thickened [40]. In a study by Spangler et al, (1978), most of the macaques had kidneys with features of tubular epithelium atrophy and the collecting tubules had both granular and cellular casts [41], or even haemozoin, protein and RBC casts in a study by Chen et al, (2001) [40], while few of the macaques had ATN with features of pyknotic nuclei and vacuolated cytoplasm [41]. There were also macaques which suggestive of ARF due to the accumulation of haematopoietic cells in the vasa recta [41], and the macaques which presented with distinct and hypercellular glomeruli due to the mild proliferation of the endothelial, epithelial and possibly mesangial cells, and exudation in capsular space [40, 41].

In severe human malaria cases, acute renal failure (ARF) is a common finding [62], which may also lead to mortality [51]. The clinical phenotype of the haemoglobinuria of "blackwater fever" correlated to renal failure has been documented as well in *P. knowlesi* malaria [63]. ATN-associated features are also relatable to the renal impairment presented in severe *P. knowlesi* malaria cases [7]. Almost all patients with severe *P. knowlesi* malaria were associated with ARF [64]. While a pooled data pointed out that almost half of severe *P. knowlesi* malaria cases were associated with ARF and 42% of the cases were linked to death [29].

POINTS TO CONSIDER

There are no remarkable chronic inflammatory reactions to the brain, heart, liver, kidney and spleen in the fatal, human *P. knowlesi* malaria as compared to the falciparum malaria, particularly in the lungs [38]. Perhaps, it can be correlated to the different immune system activation in each of the malaria species infections. The pathophysiology might differ depending on the hosts as well, where the chronic inflammatory reactions are more associated with the macaques compared to the human [38-41, 45, 46]. Although the coma was absent, the cerebral pathology of human *P. knowlesi* malaria is identical to the *P. falciparum* infections except for the absence of the visible thrombi and platelet clumping formation, and the rosetting of nRBC and pRBC, which were interspersed instead [38]. It is baffling as the ICAM-1, which is critical for the pRBC sequestration, was not indicated in *P. knowlesi* malaria yet the sequestration is still present in the brain and other organs infected with *P. knowlesi* [38]. These pathological outcomes are suggestive of other potential virulence factors or pathways of *P. knowlesi* that are yet to be fully explored, or perhaps due to both SICAvar and KIR genes unique to *P. knowlesi* [30, 65]. Onditi et al, (2015) demonstrated the possible placental malaria in the *P. knowlesi* malaria, where the placenta of the non-human primate model presented with the infiltrated pRBC and inflammatory cells, which further suggestive of cytoadherence of the cells in the placenta [66].

Additionally, histopathological changes particularly in the brains, livers, heart and the lungs of the *P. knowlesi* malaria are also demonstrated to be associated with the parasite infection rate, which could be possibly related to the parasitaemia level, but such changes are not remarkable in the spleens and kidneys of the experimental macaques [40]. It is fascinating that experimental *P. knowlesi* malaria in macaques are presented either as a mild infection as a controllable parasitaemia or a severe infection following the progression of the parasitaemia level [45].

CONCLUDING REMARKS

There are still many factors that are yet to be discovered on the pathophysiology of *P. knowlesi*, particularly in severe infection. More research is required to compare the severe *P. falciparum* malaria with other malarial manifestations, and the possible host switch events of *P. knowlesi* from the macaques into humans. For example, the utilisation of human-on-a-chip or organ-on-a-chip might be feasible to assess the pathophysiological manifestation of *P. knowlesi* with reduced invasive procedures, and the transcriptomic studies of the *P. knowlesi*-infected patients at different stages of infection. Last but not least, the lack of studies on immunology led to the difficulty in interpreting various immunopathological developments of *P. knowlesi* malaria compared to falciparum malaria.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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