Plasmodium Knowlesi: Are we Missing the Diagnosis?

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ABSTRACT

Till recently human malaria was thought to be caused by four plasmodial pathogens named falciparum, vivax, ovale and malariae. However the beginning of 21st century is characterized by the emergence of new malaria Plasmodium pathogen of simian origin (Plasmodium knowlesi), which now represents the fifth human malaria parasite. P. knowlesi can cause spectrum of illness in human beings ranging from mild, severe to fatal disease. Recently, numerous reports have described human malaria caused by Plasmodium knowlesi, which usually infects macaque monkeys. Hundreds of human cases have been reported from Southeast Asian countries. Peripheral smear examination which is gold standard for detecting malaria can misguide as P. knowlesi can resemble P. falciparum or P. malariae. Polymerase chain reaction is of value for diagnosis of P. knowlesi infection. P. knowlesi malaria is presently sensitive to majority of anti malarial drugs used to treat other plasmodial infections. Fortunately not a single case has been reported so far from India, but our country has all the factors which are conducive for occurrence of this simian malaria and hence it is necessary that Indian clinicians also become aware of this newly emerged plasmodia pathogen. This review describes the epidemiology, important morphological features of this parasite and clinical manifestations, investigations and management of malaria caused by P. knowlesi. Also it describes why India can become hub of this infection in future.

INTRODUCTION

Medical fraternity globally is well aware of the four plasmodial pathogens causing human malaria named falciparum, vivax, ovale and malariae. However since beginning of the third millennium there has been emergence of a novel malaria Plasmodium pathogen of simian origin (Plasmodium knowlesi), which now represents the fifth human malaria parasite. Recently, numerous reports have described human malaria caused by Plasmodium knowlesi, which usually infects macaque monkeys. Hundreds of human cases have been reported from Malaysia, several cases have been reported in other Southeast Asian countries, and a few cases have been reported in travelers visiting these areas. Similar to P. falciparum, P. knowlesi can cause severe and even fatal cases of disease that are more severe than those caused by the other Plasmodium species. Polymerase chain reaction is of value for diagnosis because P. knowlesi infection is easily misdiagnosed as less dangerous Plasmodium malariae infection with conventional microscopy. P. knowlesi infection should be suspected in patients who are infected with malaria in Southeast Asia. [1]

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HISTORICALASPECTS

Story of P. knowlesi started long since 1932, when blood of a monkey having P. knowlesi infection was inoculated in a patient suffering from neurosyphilis as 'human malaria therapy' but later discontinued because of life threatening infection in recipient. This experiment was carried out in India by Knowles and Das Gupta [2] Although it was known that humans could be infected with P. knowlesi by blood passage since 1932, it was not until over 30 years later that the first case of human infection by mosquito-bite under natural conditions was reported .The earliest report of a confirmed natural infection of P. knowlesi in humans was in 1965 in a US traveler who spent a few weeks in a forest of Pahang, Peninsular Malaysia [3,4.] Then for many years no human cases were reported.

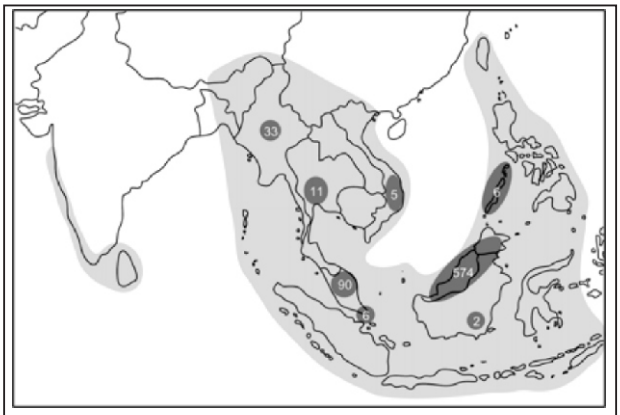
DETECTION OF LARGE FOCUS OF P.KNOWLESI IN MALAYSIA

For the first time a large focus of P. knowlesi infections

in humans was reported by Singh et al in 2004 from Malaysian Borneo [5.] These investigators noted that there had been an unusual increase in the incidence of P. malariae cases in the central divisions of Sarawak, which were later identified as P. knowlesi by the nested polymerase chain reaction (PCR) that was developed by the investigators .Identification of more cases in the following years were noted from different hospitals in Sarawak [6.] Vythillingam et al. (2008) established that the entire Malaysian Borneo has P. knowlesi cases. He reported cases from Peninsular Malaysia.[7]

Following the report of Singh et al. (2004), naturally acquired human P. knowlesi infections were reported from several South-east Asian countries namely, Thailand, Philippines, Singapore, Vietnam and Cambodia. Naturally acquired cases were also reported in travelers who visited endemic areas and went back to their native countries. Such case reports were from China in 2006, Sweden in 2006, Finland in 2007 and in New York, USA in 2008, Spain & Australia in 2010 All these travellers had history of visiting one or other South-east countries. All these reports suggest that P. knowlesi infections in humans are wide spread in South-east Asia [5,8.]

Figure 1



Plasmodium knowlesi infections reported in humans in Southeast Asia(modified from Cox-Singh and Singh). The gray area indicates the geographic distribution of *Anopheles leucosphyrus* group mosquitoes. The figures represent numbers of patients with a reported infection due to *P. knowlesi*.

EPIDEMIOLOGY

Human cases are presently confined only to Southeast Asian countries or in the travellers visiting forest areas in these countries. Highest number of cases so far has been reported from Malaysia [5,6,8.]

The natural hosts of this simian malaria parasite are the long-tailed (Macaca facsicularis) and pig-tailed (M. nemestrina) macaque monkeys and langurs (Presbytis sp.) that are distributed throughout much of Southeast Asia. However because of absence of mosquito vector in urban areasthe risk of acquiring infection is very low in urban areas. Humans can acquire infection when they visit the forest habitat of monkeys where mosquito vectors are present in abundance [9]

VECTOR

An Infected Anopheles leucosphyrus mosquito is the vector that transmits P. Knowlesi from monkeys to humans. This is forest dwelling mosquito typically lives in forest areas in south East Asia. It prefers to feed outdoor after dusk and is equally attracted to humans and monkeys .With a greater clearing of forest areas for farmland, more humans are increasingly becoming exposed to this vector. Another species of mosquitoes, Anopheles crucens has been purportedly cited as a vector of malaria knowlesi. Both species of mosquitoes have been to contain as many as 1,000 sporozoites suggesting that they are efficient vectors Anopheles lateens has also been cited as the vector for malaria [1,7,9.]

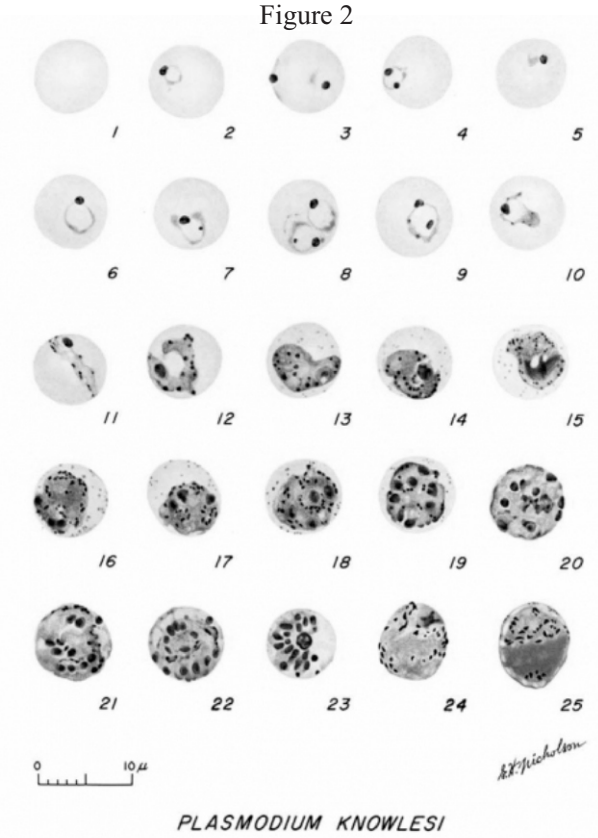
LIFE CYCLE OF P. KNOWLESI

Life cycle of P. knowlesi include an obligate sojourn in an Anopheles mosquito vector where sexual reproduction takes place. Female Anopheles injects sporozoites which get directly to the liver. Hepatic stages include schizonts that develop to release merozoites. Merozoites invade Red blood cells. It's not yet clear which RBCs (young or mature) are affected. This erythrocytic phase is of 24 hours as contrast to P. falciparum and vivax in which it is of 48 hours .Asexual stages seen in blood films are young trophozoites also calls ring forms, mature trophozoites and dividing schizonts that yield 10 to 16 merozoites for a new generation .Sexual stages in blood are microgamets (male) and macrogamets (female). Hypnozoite stage is absent in P. knowlesi infection [10.] **Figure 2**

CLINICAL FEATURES AND LABORATORY FEATURES OF P.KNOWLESI MALARIA

According to the largest studies which were undertaken in Saravak, Malasia, following clinical features were associated with knowlesi malaria Symptomatology of knowlesi infection resembles to that of any nonspecific infectious illness and is more or less similar to other plasmodial infections.

Figure 2



The stages of P. knowlesi 1.Normal RBC 2.-15. Trophozoites 16-23. Schizonts 24. Mature macrogametocyte 25. Mature microgametocyte Illustrations from: Coatney GR etal The primate malaria, Bethesda U.S. Department of Health, Education and welfare 1971.

Fever with chills and rigors are the most dominant feature. P. knowlesi requires 24 hours to complete its asexual erythrocytic cycle, which results in a unique quotidian type of fever pattern different from that for the other 4 human malaria species. However, this fever pattern may not be observed during the early phase of infections, and mixed species infections would further complicate pattern recognition related to febrile symptoms other commonly observed symptoms are headache, myalgia, arthralgia, malaise and poor appetite. Cough and abdominal pain was also seen half of the cases. Gastrointestinal symptoms in the form of anorexia, nausea, vomiting, diarrhoea and abdominal pain were also seen in significant number of cases. Amongst the physical findings notable features were raised axillary temperature, tachypnoea, tachycardia, hypotension. Hepatomegaly and splenomegaly were seen relatively in few numbers of cases.[5,9]

Complications:[9,11]

P. knowlesi has a 24-hour asexual life cycle, the shortest observed, thus far, for human-infecting parasites. This short cycle can lead to rapid increases in parasitemia and can lead to severe disease including fatalities as reported in recent studies. Patients can develop low oxygen saturation, tachypnoea chest crackles suggestive of acute respiratory distress syndrome.[9]

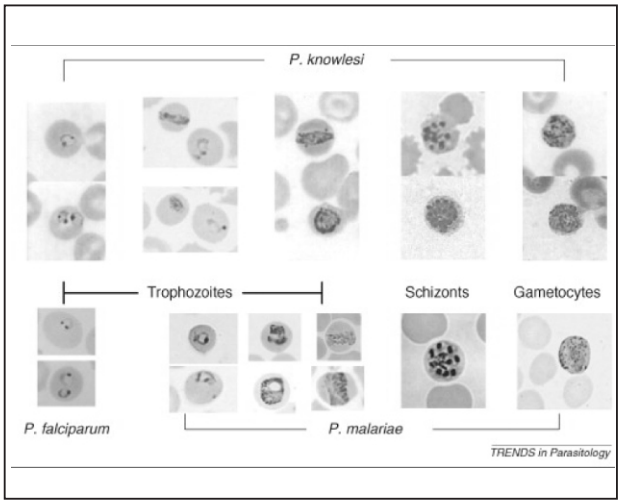
Coma and sudden death or other symptoms of cerebral involvement have been reported. However no case fulfilling WHO criteria of cerebral malaria have been noted so far.

Thrombocytopenia was almost universal feature while prolonged PT and PTT was seen in few patients. However bleeding manifestations are not seen .Severe anaemia is not commonly observed unlike to falciparum malaria. Hyperbilirubinaemia and acute renal failure are also dominant features. Hypoglycaemia and metabolic acidosis are also frequently seen .There can be single organ dysfunction or multi-organ dysfunction. Paracitaemia is a strong predictor of complications.

DIAGNOSIS

1. Giemsa stained blood film with thin and thick smears: It is often misleading as early trophozoite stages of P. knowlesi are morphologically identical to P. falciparum. The later blood stages are similar to those of P. malariae.[9] as revealed in figure 3

Figure 3



2. Rapid diagnostic tests (RDT): An antibody specific for P. knowlesi would be optimal for diagnosing P. knowlesi infection. But presently no such test is available which can detect antibody specific to P. knowlesi infection. Indirectly P. knowlesi infection can be suspected by using presently available RDT which are based on monoclonal antibodies to plasmodium lactate dehydrogenase (pLDH). Using this panel of antibodies, Thomas F. McCutchan etal showed that P. knowlesi can be distinguished from other plasmodial spp. P. knowlesi binds to both the "falciparum- specific" (17E4/7G9) and the "vivax-specific" (11D9/13H11) antibodies. Furthermore, P. knowlesi does not react antibodies specific for malariae or ovale spp. They proved that pLDH antibodies that detect P. falciparum and vivax can also be used to detect and distinguish P. knowlesi. The 1 major caveat is that a P. "knowlesi infection cannot be distinguished from a mixed infection with both P. vivax and P. falciparum in the blood.[12]

3. Polymerase chain reaction (PCR) based assay: The emergence of Plasmodium knowlesi in humans, which is in many cases misdiagnosed by microscopy as Plasmodium malariae due to the morphological similarity has contributed to the needs of detection and differentiation of malaria parasites. At present, nested PCR targeted on Plasmodium ssrRNA genes has been described as the most sensitive and specific method for Plasmodium detection. However, this method is costly and requires trained personnel for its implementation. It is also found to cross react with P.vivax in recent study.[13]

Another PCR based technique is Loop-mediated isothermal amplification (LAMP), a novel nucleic acid amplification method was developed for the clinical detection of P. knowlesi. LAMP is also an easy, convenient and cost-saving method, which only requires simple laboratory apparatus such as water bath or heating blocks to perform the test. Hence this test can also be used for detecting P. knowlesi infection.[14]

TREATMENT

Following are the guidelines for treating knowlesi malaria as per the studies undertaken at Kapit hospital, Sarawak, Malaysia. For uncomplicated malaria T chloroquine total dose of 25mg/kg , administered as 10mg/kg followed by 5mg/kg at 6, 24 and 48 hours . As per guidelines for other plasmodial infections primaquine as gametocidal drug doesn't seem to necessary as gametocytes are presently sensitive to chloroquine and hypnozoite stage is absent in knowlesi malaria.[9]

Currently there are no guidelines for treating severe knowlesi malaria, it is suggested that patients which fulfil criteria of WHO definition of severe malaria should be treated on lines of severe falciparum malaria.

P. knowlesi is sensitive to chloroquine, quinine, mefloquine and other conventional antimalarials[9]

THREAT OF P.KNOWLESI TO INDIA

Of the four countries, Bangladesh, Bhutan, Myanmar and Nepal, that border India, Myanmar is endemic for P. knowlesi. Four North-eastern states in India that border Myanmar are Arunachal Pradesh, Manipur, Mizoram and Nagaland. Travelers from Myanmar and other endemic countries visit India and people from India visit many of the endemic countries as tourists. Thus, there will be ample occasions for the P. knowlesi infections to come to India. With reference to anopheline vectors, there are three species belonging to the Leucosphyrus Group that are found in India. In forest, forest fringe and foot hill areas of North-eastern states, An. baimaii (a major vector of human malaria) and An. elegans in hill forest areas of Karnataka and Tamil Nadu in southern India (both are members of the Dirus Complex), and An. mirans (a member of the Hackeri sub group) in western-ghats in south-western region are found. An. baimaii is also a vector of human malaria parasites in Myanmar. In the North-eastern states where An. baimaii is found, neither P. knowlesi nor any other simian malaria parasites have been reported so far. Also there are no natural simian hosts for P. knowlesi in India. Thus, chances of monkey–mosquito–monkey, and human–mosquito–monkey transmission cycles to occur are remote in north-eastern states where An. baimaii is present.[5]

SUMMARY

P. knowlesi malaria, which has emerged as 5th plasmodial pathogen causing malaria, is primarily a zoonotic disease which has pigtailed and long tailed macaques as the natural hosts. Ongoing ecological changes resulting from deforestation, with an associated increase in the human population, had enabled this pathogenic species of Plasmodium to switch to humans as the preferred host. Till date, there

have been no reports of P. knowlesi malaria from India. [8, 16] But we have the vectors anopheles [17] and the primate hosts (pigtailed macaques) in the Northeastern part of India. As P. knowlesi can be easily misdiagnosed as P. malariae or P. falciparum by light microscopy, we might be missing cases of P. knowlesi malaria in India. Therefore, it is necessary to supplement light microscopy with other advanced diagnostic tests like PCR which will definitely help us to detect cases of P. knowlesi. Apart from these improved diagnostic facilities, it is mandetary to create an increased awareness amongst the clinicians and microbiologist to detect any new infection due to P. knowlesi in India. [16]

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