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Case Report

Mixed Infection due to *Plasmodium malariae* and *Plasmodium vivax*: A First Case Report from Rural North India

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ABSTRACT

Mixed malaria infections often go unrecognized and are therefore under reported. We hereby report the first described case of mixed infection due to *P. malariae* and *P. ovale* from most backward region of country emphasizing possibility of change in epidemiological pattern of malaria in the region. Also present case report highlight the increasing importance of Polymerase Chain Reaction in diagnosing malaria especially mixed malaria.

Key words: Malaria, *Plasmodium malariae*, *Plasmodium falciparum*, Polymerase Chain Reaction in diagnosing.

INTRODUCTION

Malaria is one of the most important parasitic infection throughout the world and it results in high mortality rates. The causative agent *Plasmodium* spp. are transmitted by Anopheles mosquitoes.[1] Human malaria is a protozoan infection caused by five *Plasmodium* species which includes *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. All species cause the disease which is different in transmission, nature of immune response, clinical manifestation and response to drugs.[2-4] National vector borne disease control program estimate about 2 – 3 million cases of malaria occur annually in India.[5]

The most prevalent species causing disease in India is *P. vivax* accounting for about 65% of cases and next common species is *P. falciparum* which is responsible for about 35% of cases. The remaining species are infrequently seen in India with few cases sprouting from different states of India such as Orissa, West Bengal, Madhya Pradesh, Karnataka, Tamil Nadu, Kerala, Arunachal Pradesh and Assam.[2,6-10] *P. ovale* is the rarest infection with only four cases reported in India, one each from Kolkata, Orissa, Delhi and Assam.[11] To best of our knowledge, we are reporting probably the first case of mixed infection by *P. malaria* and *P. vivax* from Haryana.

CASE REPORT

A 50-yr old man from Nuh, Haryana walked in outpatient department with complaints of fever, headache and abdominal pain. There was no history of travel to other state of India. On examination, pallor was present, abdomen was soft with diffuse tenderness. Other systems were within normal limits He was evaluated for fever. Laboratory tests showed Hemoglobin - 9 gm/dl. Other parameters were within normal limits. Thick and thin blood smears were prepared. Microscopic examination of blood smears showed band form, ring forms and gametocytes of malarial parasite. (Figure 1). On microscopy a diagnosis of *P. malariae* was made due to the size of infected RBCs being same as that of uninfected ones. Also presence of malarial gametocytes were observed. On the basis of these findings, a diagnosis of Malaria due to *P. malariae* was made. Since the presence of *P. malariae* in this region was not reported before, it was decided to send the sample to National Institute of Malaria Research, New Delhi for confirmation. Polymerase Chain Reaction (PCR) was used for confirmation and presence of *P. malariae* along with *P. vivax* was confirmed in the blood sample. The results underline the possibility of a co-infection by the two species. The gametocytes of two species resemble each other so probably missed on microscopy. The patient was treated under supervision for uncomplicated malaria with oral artesunate plus sulphadoxine-pyrimethamine for three days. Fever subsided on the 3rd day after the initial examination, the daily dose continued for seven days and the patient remained afebrile until the day of discharge.

DISCUSSION

This is probably first case report of *P. malariae* from Haryana which was also confirmed by PCR. The estimated prevalence of *P. malariae* - *P. vivax* mixed infections is documented to be less than 3%.[12]

During microscopic examination of thick blood film, the morphology of *P. malariae* can be confused with *P. vivax*. The ring forms of the two species are so similar that Garnham described the ring forms of *P. malariae* in blood films as "rather like those of *P. vivax* although less amoeboid and with a more dense

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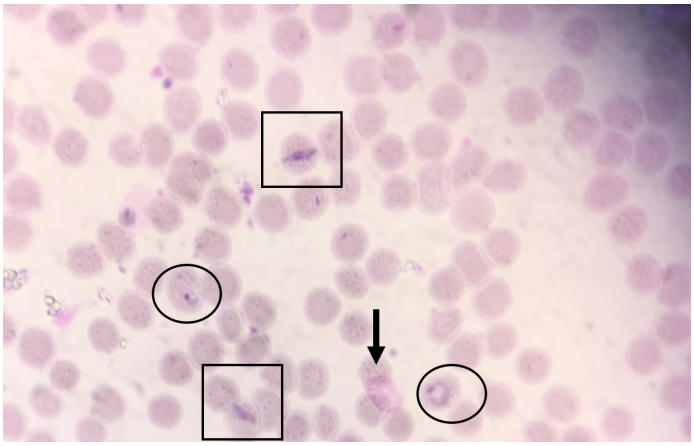

Figure 1: Band forms and Ring forms (Size of infected RBCs is same that of uninfected) and Gametocytes of *P. malariae*. Magnification 10x100

ring of cytoplasm". However, size of infected RBCs can be crucial while arriving at a diagnosis. Under NVBDCP, Jaswant Singh and Bhattacharyya (JSB) stain is used for routine malaria microscopy examination of thick and thin smear. The morphological changes induced by hemolysis can hamper the identification of infecting species or inadequate knowledge and experience of the reporting personnel to identify malaria parasites other than *P. falciparum* and *P. vivax*, diagnosis of atypical parasites are missed. Moreover, *P. malariae* and *P. ovale* species are difficult to identify if the typical morphology of the infected host cell is damaged. On microscopy *P. malariae* in India remains underestimated. PCR based identification method is a confirmatory diagnostic tool. But the facility remain largely lacking in rural and remote areas like ours.[2]

P. malariae is known for its recurrent infection potential and propensity to develop nephrotic syndrome in patients. *P. malariae* can remain for decades within a human host and in a state potentially infectious to mosquitoes thus, facilitating the transmission of this species over a long period of time.[2,13]

P. malariae rapidly responds to routinely used antimalarial drugs and is not known to cause severe clinical disease. Our patient also responded well to the treatment. To control malarial infection, successful treatment is required, as incomplete treatment can lead to the development of resistance and recrudescence. Choloroquine drug resistance is common in Plasmodium spp. Now artemisinin-based combination therapies are used for *P. falciparum* and mixed malaria infections. This regimen consists of doxycycline 100 mg/BD and artesunate 2.4 mg/kg, intravenously at 0, 12 and 24 hr, then daily for seven days and the addition of primaquine 30 mg/day on the third day, for a period of three days, to eradicate the gametocytes. Mixed malaria species infection is common, but to control the disease early diagnosis and appropriate treatment is required.[1]

CONCLUSION

Although *P. malariae* do not cause sever disease but its presence in this part of country indicates change in epidemiological pattern of malaria infection. This region of Haryana is endemic for malarial infection and complicated cases of cerebral malaria are not uncommon. Even though the morphology was quite characteristic of *P. malariae*, making diagnosis possible, on PCR simultaneous presence of *P. vivax* was also confirmed. Geographical region should not be deterrent for doing PCR; as dynamics of human movement in an area can spread the infection.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

PCR: Polymerase chain Reaction; **JSB:** Jaswant Singh and Bhattacharyya; **ICMR:** Indian Council of Medical Research.

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