Original Article

Presence of Malaria Parasite in Vivax Malaria Patients after Completion of Anti-malaria Therapy in a Tertiary Care Hospital of Kolkata : A Real World Observational Study

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Background : Malaria is one of the most prevalent protozoal diseases in the world caused by the genus Plasmodium. The disease is transmitted to humans through the bites of infected Anopheles mosquitoes and is widespread in tropical and subtropical regions around the Equator. Despite efforts to eradicate the disease, global malaria cases are on the rise again and delayed parasite clearance and relapse are major hindrances to malaria elimination. The only drug that can prevent these and provide radical cure are the 8-aminoquinolines, but they are underused due to the risk of oxidant hemolysis in patients with G6PD deficiency.

Materials and Methods : The study was conducted to assess the persistence of parasites in peripheral blood smear, the level of G6PD in blood and the markers of severity of Plasmodium vivax infection in patients diagnosed with vivax malaria attending MOPD, Medical College and Hospital, Kolkata, India. The study was a prospective observational study, including proper history taking, collection of blood samples, and monitoring of peripheral blood smears after day 3, 2 weeks (day 14) and 6 weeks (day 42) from the initiation of antimalarial therapy.

Results : The results showed that out of 110 patients, 9.1% had parasites in the peripheral blood smear. The mean G6PD level was significantly lower in patients with a positive parasite smear than in those with a negative smear. Additionally, markers of severity of Plasmodium vivax infection were also assessed and the results suggest that thrombocytopenia is a strong marker of severe vivax malaria.

Discussion & Conclusion : Overall, the study highlights the importance of monitoring parasite clearance and relapse in vivax malaria patients and the need for careful consideration of G6PD deficiency status before administering primaquine. It also emphasizes the importance of monitoring the markers of severity of Plasmodium vivax infection to identify patients at risk of developing severe malaria.

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Key words **: Plasmodium vivax, Radical Cure, Primaquine, G6PD Deficiency.**

Malaria, caused by the genus Plasmodium, is one of the most prevalent protozoal disease in the world. It is one of the few diseases in which morbidity is measured in hundreds of millions of cases per year[1]. The disease is widespread in the tropical and subtropical regions that exist in a broad band around equator. Malaria is transmitted to humans through the bites of infected Anopheles mosquitoes. Five parasite species namely P vivax, P falciparum, P malariae, P ovale and P Knowlesi cause malaria in humans. Among them, two of these species-P falciparum and P vivax-pose the greatest threat and account for more than 90% of the total malaria cases worldwide. Based on recent trends and outcomes, global malaria cases are on the rise again with increasing mortality from the severe manifestations of malaria, raising concerns of a resurgence of this too often deadly disease[2].

Efforts to eradicate malaria have clearly been unsuccessful in many regions of the world and current

Editor's Comment :

- Persistence of Malaria Parasites Post-Therapy : A significant finding was that 9.1% of patients still had malaria parasites in their blood after completing antimalarial therapy, indicating the need for effective post-treatment monitoring.
- Importance of G6PD Level Testing : The study emphasizes the importance of G6PD deficiency testing, as lower G6PD levels were significantly associated with the presence of parasites post-treatment. This has implications for safe and effective primaquine use.
- Significance of Severe Malaria Indicators : The research highlighted thrombocytopenia as a strong marker for severe vivax malaria, underscoring the need for careful monitoring of severe malaria indicators in patients.

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efforts to control the disease focus on reducing attributable morbidity and mortality. Delayed parasite clearence and relapse are major hindrance to malaria elimination, because the only drug that can prevent them and thereby provide radical cure are the 8-aminoquinolines and they are underused. In patients with G6PD deficiency, this drug causes oxidant hemolysis which is very common in tropical areas. Testing of G6PD is often unavailable, so prescribers are commonly reluctant to risk hemolysis in order to prevent relapse. The clearence of malaria parasites from blood and reduction in the probablity of relapse depends on the dose, the quality and bioavailability of the drug 8 aminoquinoline (primaquine) and the number of activable hypnozoites in liver.

Clinical efficacy is defined by the clearence and recurrence of parasitemia. In most patients these occur at relatively low level of patency and thus expert microscopy is crucial in the assessment of blood films. Parasitemia clearing within 4 days and not reappearing in 35 days may be defined as chloroquine sensitive malaria. There is no evidence of resistance to therapeutic doses of Primaquine by hypnozoites of P vivax. A loss of function cyt P 450 2D6 genotype resulting in inadequate metabolism of Primaquine is an important cause of therapeutic failure. Other possible causes of therapeutic failure must be ruled out including insufficient Primaquine quality, reinfection after therapy.

Materials and Methods

Definition of study population : Patients diagnosed with vivax malaria attending MOPD, Medical College and Hospital, Kolkata including age more than 12 years during the study period.

Inclusion criteria : Cases diagnosed with vivax malaria by thick/thin smear or rapid diagnostic kit encompassing ages more than 12 years.

Exclusion criteria :

(1) Patients with malaria infection other than vivax and Mixed infection (vivax plus falciparum)

(2) Malaria with other bacterial or viral coinfecion

(3) Pregnant patients

Sample size calculation : Assuming 1.5% of the patients attending our OPD are suffering from P vivax. 5% presision and 5% alpha error, the required sample size was 118(~120). We have collected data from 110 patients. Due to COVID-19 pandemic, study population inclusion was restricted.

Study Design : Prospective Observational Study

Study procedure : Our study was a prospective observational study. It included proper history taking, collection of blood samples and monitoring of peripheral blood smears after day 3, 2 weeks (day 14) and 6 weeks (day 42) from the initiation of antimalarial therapy. We intended to look for the presence of malarla parasite in peripheral blood smear of those patients, the level of G6PD in blood and also assess the markers of severity of Plasmodium vivax infection.

Results

In our study, 45 (40.9%) patients were female and 65 (59.1%) patients were male and 15 (13.6%) patients were from Rural area and 95 (86.4%) patients were from Urban area (Table 1).

Table 1 — *Distribution of Parasite of Peripheral Blood Smear on follow up*

Parasite of Peripheral Blood Smear	Frequency	Percent
Absent	100	90.9%
Present	10	9.1%
Total	110	100.0%

In our study, 10 (9.1%) patients had Parasite of Peripheral Blood Smear. Table 1 had depicted the presence or absence of parasites in peripheral smear.

Table 2 had shown G6PD deficient status and persistence of parasites in peripheral smear. In patients not having parasites in peripheral blood smear, the mean G6PD Level (Mean±SD) of patients was 14.8570± 3.2146 and patients with parasites in peripheral blood smear, the mean G6PD Level (Mean±SD) of patients was 8.1900±4.4859. Distribution of mean G6PD Level with parasite positive peripheral blood smear was statistically significant (p<0.0001). Fig 1 had shown the distribution of mean G6PD Level with presence or absent of parasites in peripheral blood smear.

Discussion

Our study was done to look for the rate of parasite clearence for peripheral blood smear of Plasmodium vivax malaria. In case of Plasmodium vivax malaria, following reasons are there for which malaria parasite may presenet in peripheral blood smear after completion of anti-malarial therapy. First of them is,drug resistance malaria. Kiran K Dayananda, *et al*[4] have discussed about drug resistance malaria. Nicoholas J, *et al*[5] study shows anti malarial drug effect on parasite dynamics in vivax malaria. Second

Table 2 — *Distribution of mean G6PD Level: Parasite of Peripheral Blood Smear*

		Number	Mean	SD	Minimum	Maximum	Median	pvalue
G6PD Level	Absent	100	14.8570	3.2146	8.3000	20.1000	15.1000	<0.000
	Present	10	8.1900	4.4859	4.3000	17.1000	6.2000	1

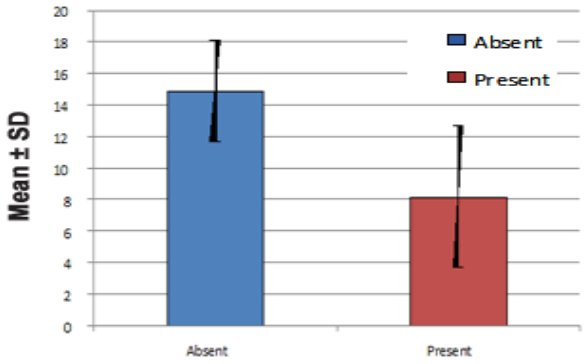

Fig 1 — Distribution of mean G6PD Level with presence or absent of parasites in peripheral blood smear

important reason for delayed parasite clearance is severe vivax malaria. Rishikesh Kumar, *et al*[6] shows how severity of malaria affect the parasite clearence. T Angel Miraclin, *et al*[7] study found slow parasite clearance. Now in case of recrudesence or relapse, which is not very uncommon in case of vivax malaria, we may found malaria parasite in peripheral blood smear after completion of therapy. Brian Greenwood, *et al*[8] study shows vivax malaria recurrence in Brazil.

In our study, a total of 110 patients were studied over a period of one and a half years. Among them 10 patients have malaria parasite in peripheral blood smear after completion of anti-malarial therapy. Male population was higher than the female population and we found that, most of the patients were 31-40 years old. 9.1% patients had Parasite in Peripheral Blood Smear and mostly observed in patients from urban population which was not statistically significant. It was shown that malarial parasites were found significantly in the blood smear of the patients who are receiving chloroquine alone than the group of patients who was receiving both chloroquine and primaquine. G6PD Level was significantly decreased in patients with Plasmodium vivax in peripheral blood smear after completion of antimalarial therapy. The parasite clearance was hastened in the patients who were receiving primaquine for radical cure. G6PD testing should be made widely available so that primaquine can be given more safely. Though our study has few limitations like the number of study population is less and we did not take any parameters to differenriate reinfection from relapse.

Conclusion

Presence of malaria parasites in peripheral blood smear of Plasmodium vivax patients after successful anti-malarial therapy is not uncommon. Use of G6PD level estimation widely and using primaquine are important steps to follow for successful therapy and need to be available and accessible widely.

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