

POSSIBLE IMPACT OF MUTATIONS AGAINST ANTIMALARIALS ON PHENOTYPIC EXPRESSION OF *PLASMODIUM FALCIPARUM* AND *Plasmodium vivax*

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ABSTRACT

Polymorphism is observed in the Signet ring stage of *Plasmodium falciparum* and *P. vivax*. In 5-10% patients of Sudan, Yemen, Ethiopia and Jazan of Saudi Arabia, rings were of very small size ranging from 0.7-2 μ both round and oblong with cytoplasm of non-uniform thickness, while in 30% patients, rings were slightly oblong and bigger (i.e. 2-3 μ) with non-uniform thickness. Alteration in size of *P. vivax* ring was also observed in about 10% patients in whom rings were slightly smaller (2-3 μ) than that of its usual size showing resemblance with *P. falciparum* ring. Variations in ring shape were also observed in 10% *P. falciparum* cases in the patients of Bangladesh, Pakistan and India. This polymorphism in *Plasmodium* with different phenotypic expressions seems to be due to the mutations against anti-malarials, frequently used to treat resistant cases of malaria.

Key words: Mutation, anti-malarial, phenotypic expression, *Plasmodium*

INTRODUCTION

Malaria, a disease of tropics remains one of the leading causes of morbidity and mortality. Malaria is endemic in about 91 countries and claims 2-3 million lives annually (Singh et al., 2004). Available data indicate dramatic increase in *Plasmodium falciparum* infection in many countries including India and is a matter of concern. Emerging resistance to chloroquine by *Plasmodium* threatens the health of hundreds of millions of people exposed to the risk of infection with this parasite. As chloroquine resistant parasites have spread in many countries including India, Pyrimethamine -Sulfadoxine (PM-SD) has increasingly become the drug of choice for the treatment of uncomplicated malaria. The emerging PM-SD resistance is of immediate concern since it is an affordable antimalarial drug for poor countries. Microscopic examination of thin blood smear is the routine method for specific diagnosis in majority of the hospitals, clinics and diagnostic centers. It is observed that a fair percentage of technicians are not able to differentiate between *P. falciparum* and *P. vivax* because of lack of expertise and poor quality of microscopes provided to them. Treatment depends on the technician's report and in case of confusion *P. falciparum* is declared. In such cases antimalarials like Fansidar, Metakelfin, Mefloquin or Artemisine derivative are

prescribed by the clinicians in order to avoid any risk on the life of patient which finally leads to the development of drug resistance by frequent dosing of these antimalarials, whether it is *P. falciparum* or *P. vivax*. In the recent past, varying degrees of mutations against sulfadoxine-pyrimethamine combination in *P. falciparum* and *P. vivax* have been reported in India and abroad (Bosco et al., 1998; Biswas et al., 2000; Nzila et al., 2000; Ahmad Das et al., 2006; Ahmad Lumb et al., 2006; Alam et al., 2007; Lumb et al., 2009). Keeping the above facts in view, an attempt is made to show varied forms of ring of *P. falciparum* and *P. vivax* along with other stages which could be visible in thin and thick blood smears and help in correct diagnosis and thereby prevent mutation by avoiding unnecessary loading of antimalarials used to treat resistant *P. falciparum* cases.

MATERIAL AND METHODS

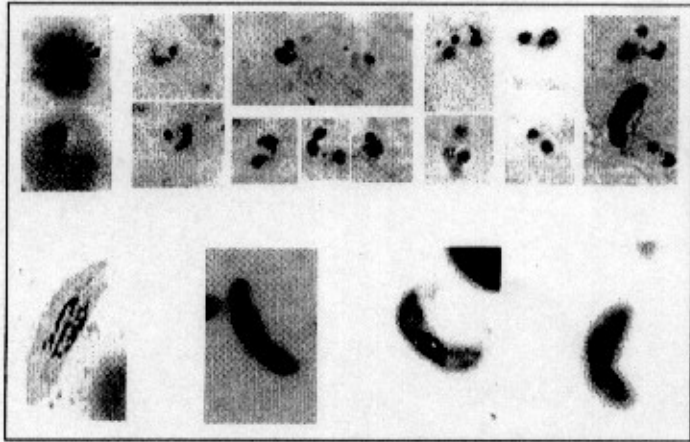
Present study was conducted from the blood smears of the patients of different nationalities during 2004-2010. The patients were from Sudan, Yemen, Ethiopia, Jazan of Saudi Arabia, Bangladesh, Pakistan and India. Thin and thick blood smears were prepared on glass slides by pricking finger of the patients and stained with JSB and Giemsa stains. In *P. vivax*, positivity was recorded after looking into different stages of the parasite such as ring, trophozoite, schizont and gametocyte. For *P. falciparum*, ring and gametocyte were the stages which were identified before declaring its positivity.

RESULTS AND DISCUSSION

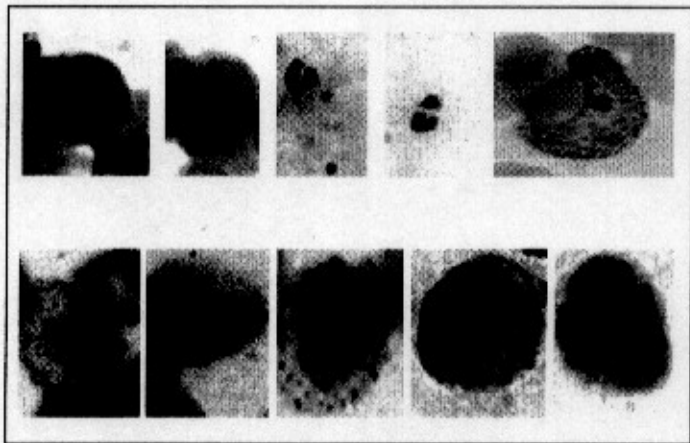
Observations made from 300 slides collected from patients of different nationalities showed variations from usual size and shape of ring specially in *P. falciparum*. Size of ring in case of this species ranged from 0.7 to 2 μ with lot of variations in their shape. The cytoplasmic portion was not uniform like that of a typical ring. In some cases, it is very small and rounded (0.7 μ) and in others it is again small but oblong (1-2 μ) (Plate 1). These types of rings were found in 5-10% cases. About 20-30 percent *P. falciparum* slides showed ring of around 2-3 μ but here too, cytoplasmic portion was not of uniform thickness and showed similarity with *P. vivax* ring in shape as it was smaller and thinner. These variations were recorded mostly in patients of Sudan, Yemen, Ethiopia and Jazan of Saudi Arabia. In Bangladeshis, Pakistanis and Indians, mostly typical *P. falciparum* and *P. vivax* stages were observed. In few slides of *P. falciparum*, variations were observed in the rings which were comparatively of bigger size than what was observed in Sudanis, Yemenis or Ethiopian patients. Male gametocytes showed scattered chromatin in the centre while female gametocytes showed compact mass of chromatin in the centre as usual.

In *P. vivax*, trophozoites, schizonts and gametocytes were almost normal in all the nationals with slight fluctuation in size. Schizonts generally showed irregular distribution of merozoites and not the typical rosette shape (Plate 1).

During the present study, it was observed that the diagnostic stages were not typically classical structures, particularly ring stages in *P. falciparum*. In resistant cases, rings were very



Different stages of *Plasmodium falciparum*



Different stages of *Plasmodium vivax*

small, round or oblong with non-uniform thickness of cytoplasmic portion, not thin and uniform like typical ring. In a few other *P. falciparum* cases, rings were large like *P. vivax* ring in shape which may cause confusion in specific diagnosis. In *P. vivax* smaller rings were observed but here cytoplasmic portion was thick with coarse chromatin mass. This difference in size and shape of ring in *P. falciparum* and *P. vivax* might be due to alteration of amino acids at different codons of Dihydrofolate Reductase (DHFR) enzyme of *Plasmodium* which resulted in drug resistance. Resistance against sulfadoxine-pyrimethamine combination is developing fast with treatment failures which are recorded in Tanzania, Kenya, Thailand and India (Bosco et al., 1998; Biswas et al., 2000; Nzila et al., 2000; Magesha et al., 2001; Mutangua et al., 2001; Ahmad Das et al., 2006; Alam et al., 2007; Lumb et al., 2009). Our recent findings have proved that mutations have taken place against sulfadoxine pyrimethamine in *P. falciparum* at different loci in different parts of the country. Normal *Plasmodium falciparum* show amino acid sequence in DHFR enzyme as N51, C59, S108 and I164. We observed single mutation in above enzyme at codon 108 as S108N and double mutations as C59R & S108N or N51I & S108N or S108N and I164L in Uttar Pradesh, India. Triple and quadruple mutations were recorded as C59R plus S108N plus I164L and N51I plus C59R plus S108N plus I164L in Assam and Car Nicobar (A&N) island, respectively (Ahmad Lumb et al., 2006; Alam et al., 2007). Similar observations regarding mutations were made by other workers in *P. falciparum* (Plowe et al., 1997; Bosco et al., 1998; Biswas et al., 2000; Nzila et al., 2000). We observed mutation in both *P. falciparum* and *P. vivax* which probably occurred because of the fact that both the species co-exist and have similar biochemical targets which were exposed during medication (Ahmad Lumb et al. 2006, Alam et al., 2007). The treatment failure occurred because of the development of resistance specially in *P. falciparum* as a result of mutation which should be the reason for the polymorphism and different phenotypic expressions of the parasite in the early stage i.e. ring stage which showed varied forms may result in confused diagnosis.

Polymorphism is observed in the ring stage of *Plasmodium falciparum* and *P. vivax* in the patients of Sudan, Yemen, Ethiopia, Jazan of Saudi Arabia, Bangladesh, Pakistan and India. Size of ring ranged between 0.7-2 μ , both round and oblong having non-uniform thickness of cytoplasm. Alteration in size of *P. vivax* ring was also observed which was slightly smaller (2-3 μ) than that of its usual size showing resemblance with *P. falciparum* ring. This polymorphism in *Plasmodium* with different phenotypic expressions seems to be due to the mutations against curative antimalarials frequently used to treat resistant cases of malaria.

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