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PREVALENCE OF *PLASMODIUM FALCIPARUM* AND *P. VIVAX* INFECTION IN MALARIA PATIENTS OF MATIRANGA, KHAGRACHHARI

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Abstract

The investigation was performed to study the different growth stages of malarial parasite found in the peripheral blood of malaria patients of Matiranga, Khagrachhari. Rapid diagnostic test (RDT) was used to screen positive patient. The early trophozoite stages were measured 1.3 - 1.6 μm, late trophozoites 2.5 - 2.9 μm in diameter, microgametes 9-10 μm by 2 - 3 μm and macrogametes 11 - 12 μm by 2 - 3 μm for *Plasmodium falciparum*. The early trophozoites 2.2 - 3.0 μm and late trophozoites 3.3 - 5.0 μm in diameter for *Plasmodium vivax* were measured. The patients of age group 1 (0 - 10 years) were more (25%) vulnerable to the severe malaria (++++), which was 10% of the total infection, while only 10% patients of age group 2 (> 10 years) were suffering from severe form, only 0.6% of the total infection. In age group 1 (0 - 10 yrs), + infection was 30%, ++ was 40% and +++ was 5%. In age group 2 (> 10 years), + infection was 26.66%, ++ 56.67% and +++ 6.67%, respectively.

Introduction

Malaria is one of the most common infectious disease and responsible for enormous public health problems. It is acknowledged to be by far the most important tropical parasitic disease(1). It has been estimated that 300 - 500 million new clinical cases of malaria (more than 90% of which in tropical Africa) occur every year worldwide causing 1.5 - 2.7 millions of deaths, majority being recorded in African Children(2). Outside Africa, other countries pay a heavy tribute to malaria. Among them Bangladesh, India, Brazil, Sri-Lanka and Afghanistan are most involved(3).

Malaria is caused by protozoan parasites of the genus *Plasmodium*. Only four species of the genus *Plasmodium* can infect human; *Plasmodium falciparum, P. vivax, P. ovale* and *P. malariae*. The most serious forms of the disease are caused by *P. falciparum* and *P. vivax*(4).

In Bangladesh, 90% of malaria episodes are due to *P. falciparum* and occur in 13 endemic districts in the northeast and southeast close to and/or bordering India and Myanmar, namely Kurigram, Sherpur, Mymenshingh, Netrokona, Sunamganj, Sylhet,

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Habiganj, Maulavibazar, Chittagong, Cox's Bazar, Khagrachhari, Rangamati and Bandarban. Ninety eight per cent of the malaria cases are reported from these 13 districts[5], whereas 70% of cases are reported from the last four districts. In these 4 districts, Falciparum malaria accounts for the major mortality and morbidity. *P. vivax* is also reported in Bangladesh[6]. *P. malariae* are very rarely reported[7].

The parasite is an intracellular blood parasite and in erythrocyte, the parasites show different forms, namely trophozoite (early and late), schizont (early and late) and gametocytes. In the four human pathogenic parasites of the genus *Plasmodium*, the forms show differences in shape, size and appearance and they also show different level of parasitemia[8].

The Malaria Eradication Program (MEP) adopted in 1961 in Bangladesh[9], later merged with PHC, adopted in 1977[10]. Revised malaria control strategy was adopted in 1994 and in 1998, piloting of Roll Back Malaria started in one of the Hill Tracts[10] drops down the morbidity due to malaria. Though the death rate dropped down but the positive cases are still seen and in a huge number also. It is important to notice that proportion of *Plasmodium falciparum* (Pf) cases has been increasing at an alarming rate since 1999 and has reached 78.5% in 2005 to 83% in 2008[11].

Under these circumstances, it is important to distinguish between different stages of malarial parasite in human peripheral blood for the proper diagnosis of malaria. Microscopy is the more reliable diagnostic process. There is no previous work related to this in Bangladesh. The present work may help in some ways to identify the malarial parasite in the blood film in diagnosing malaria by microscopy. The present investigation, also tried to find out a relationship between age group and parasitemia level of the subjects from Matiranga, Khagrachhari in Bangladesh.

Materials and Methods

The present study was carried out at the Matiranga upazila of Khagrachhari district and was conducted between June and September, 2009 in the peak malaria transmission season. Matiranga upazila was selected for adequate laboratory facilities provided by Matiranga upazila health complex and ICDDR,B.

In the present observation, 50 malaria positive patients were enrolled from an ongoing research project of ICDDR,B at Matiranga upazilla health complex. In the study, two age groups were considered: Group 1 (0 - 10 years) and group 2 (>10 years).

Fifty positive patients were enrolled for the study and Rapid Diagnostic Test (RDT) kit 'FalciVax' (Zephyr Biomedicals, India) that detects both *Plasmodium falciparum* and *P. vivax* specific antigen used to screen positive patients.

Both thick and thin smears were prepared from each of the samples. Both the smears were stained with 10% Giemsa's stain and the thin smear was fixed with 100% methanol.

The parasitemia (presence and number of parasites) was established by 'Semi Quantitative Counting Method'[12]. The system entails using a code of between one and four plus signs (+ = 1 - 10 parasite per 100 high power fields of thick film, ++ = 11 - 100 parasites per 100 high power fields of thick film, +++ = 1 - 10 parasites in every high power field of thick film, ++++ = More than 10 parasites in every high power field of thick film).

The size of the different stages of parasites was measured by an optical microscope. The measurements of different stages of the parasites were calculated from a graticule fitted with the eyepiece of the microscope.

Results and Discussion

Different stages of *Plasmodium falciparum* and *Plasmodium vivax* were observed in the peripheral blood films of the hosts. The special features of the stages for both the parasites are summarized below:

Plasmodium falciparum

- *Early trophozoite:* Mainly small and delicate rings (thin film) or small pieces of cytoplasm with chromatin dot (thick film). Double chromatin dot were observed occasionally. Ring of drug resistant strain may appear thick and distorted. In heavy infections, a few larger rings may be seen. Mainly rounded, oval shape may also be seen. It is 1.3 - 1.6 μm in diameter (Fig. 1a).
- *Late trophozoite:* These trophozoites are same as the earlier one, but with a very little difference in their appearance, they are much more distorted and the ring is a little bit larger in diameter than the former. Mainly rounded, oval shape may also be seen. It is generally 2.5 - 2.9 μm in diameter. But it may also reach a diameter of 4 μm (Fig. 1b).
- *Schizont*: This stage was not seen in our slides. This stage is very rarely seen.

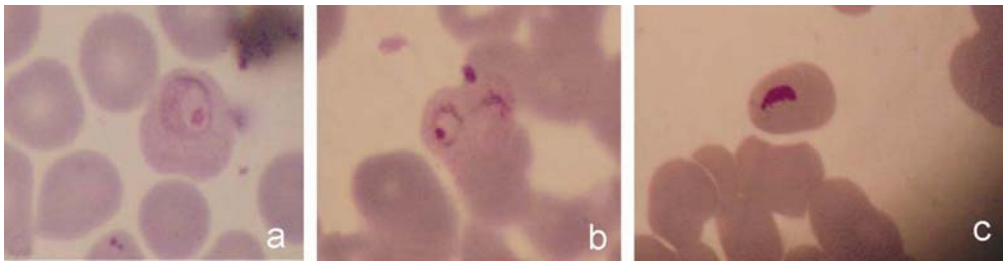

Fig. 1. Different stages of *Plasmodium falciparum.* a. Early trophozoite b. Late trophozoite c. Macrogamete.

- *Gametocytes:* Pigment granules present, mainly around nucleus. There are two types of gametes, Microgamete (male) and the Macrogametes (female). The microgametes have blunt ends and a broader outlook then the macrogametes. Cresent (banana) shaped with rounded or pointed ends but oval forms may be seen. The microgametes

have a measurement of 9 - 10 µm by 2 - 3 µm, and the macrogametes have a measurement of 11 - 12 µm by 2 - 3 µm (Fig. 1c).

Plasmodium vivax

- *Early trophozoite:* Most are large, rounded or oval and irregular in form. In thick films the cytoplasm appears fragmented. These trophozoites have the diameter of 2.2 - 3.0 µm. The cytoplasmic ring is more thicker than that of *P. falciparum*, and also much distorted (Fig. 2a).
- *Late trophozoite:* Most of them are also large, rounded or oval and irregular in form. In thick films the cytoplasm appears fragmented. These trophozoites have the diameter of 3.3 - 5.0 µm (Fig. 2b).
- *Schizont:* Large, round or irregular in form. Mature schizonts contain 24 or more merozoite and small amount of pigment. It appears as a cluster of cells in the thick film (Fig. 2c).
- *Gametocytes:* This stage is very rarely seen in the blood film for this species.

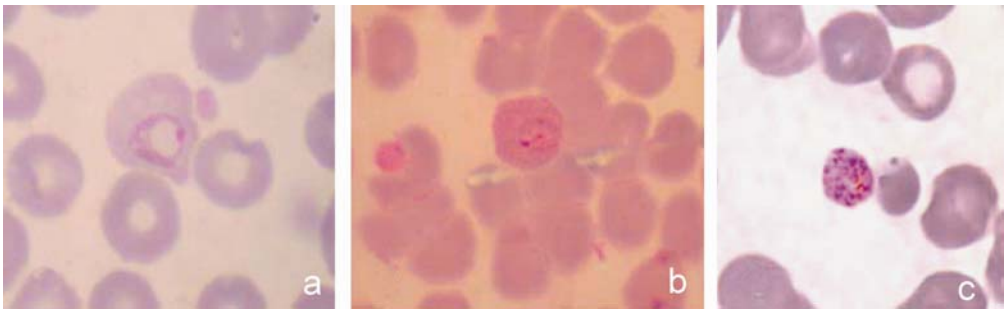

Fig. 2. Stages of *Plasmodium vivax*. a. Early trophozoite. b. Late trophozoite. c. Schizont.

Among the total patients (n = 50), 27 (54%) were male and 23 (46%) were female of the total sample population. Twenty (40%) patients are of age group 1 (0 - 10 years) and 30 (60%) patients are of age group 2 (> 10 years) (Table 1).

Among 50 infected patients, 47 (94%) were infected by *Plasmodium falciparum* and only 3 (6%) patients were infected by *P. vivax* (Table 2).

The parasitemia were counted in the 'Semi Quantitative Counting' Method. 32% (16 of 50) of the patients were suffering from + infection, 44% (22 of 50) of them were suffering from ++ infection, 8% (4 of 50) of them were suffering from +++, while 16% (8 of 50) of them were suffering ++++ infection, which is the most severe infection level (Table 3).

Parasitemia in different age groups varied. Among the 20 patients of age group 1 (0 - 10 yrs), 30% (6) were suffering from + infection, 40% (8) were suffering from ++, 5% (1) were suffering from +++, while 25% (5) were suffering from ++++ infection (Table 4).

Among the 30 patients of age group 2 (> 10 yrs), 26.66% (8) were suffering from + infection, 56.66% (17) were suffering from ++, 6.67% (2) were suffering from +++, while 10% (3) were suffering from ++++ infection (Table 4).

Table 1. Prevalence (%) of malaria in different sex and age groups.

	n = 50	Prevalence (%)		n = 50	Prevalence (%)
Male	27	54	Age group (0 - 10 yrs)	20	40
Female	23	46	Age group 2 (> 10 yrs)	30	60

Table 2. Prevalence of the malarial parasites.

Parasite	n = 50	Prevalence (%)
Plasmodium falciparum	47	94
Plasmodium vivax	3	6

Table 3. Percentage of parasitemia among the patients.

Level of parasitemia	n = 50	Prevalence (%)
+	16	32
++	22	44
+++	4	8
++++	8	16

Table 4. Percentage of parasitemia in different age groups.

n = 50	Age group 1 (0 - 10 yrs) =20			Age group 2 (>10 yrs) =30		
Level of parasitemia	n = 20	100 (%)	Percentage in the total infection	n = 30	100 (%)	Percentage in the total infection
+	6	30	12	8	26.66	16
++	8	40	16	17	56.67	34
+++	1	5	2	2	6.67	4
++++	5	25	10	3	10	0.6

Most of the patients were infected with ++ parasitemia (44%), while the lowest parasitemia was +++, only 8% (Table 3).

The age group 1 patients were more vulnerable to the severe malaria. The study revealed 25% age group 1 (0 - 10 yrs) patients were suffering from severe malaria (++++), which was 10% of the total infection, while the age group 2 (>10 years) were suffering only 10% of the severe form, amounting only 0.6% of the total infection (Table 3).

It was observed that, very little difference in the prevalence rates among male and female. Again, there was more severe (++++) infection in age group 1 (10%) than the age group 2 (6%) and the result was supported[5,13].

In the present study, the early trophozoites were measured 1.3 - 1.6 μm, late trophozoites 2.5 - 2.9 μm in diameter, microgametes 9 - 10 μm by 2 - 3 μm and macrogametes 11 - 12 μm by 2 - 3 μm for *Plasmodium falciparum*[8,12,14] and the early trophozoites were measured 2.2 - 3.0 μm and late trophozoites 3.3 - 5.0 μm in diameter for *Plasmodium vivax*[8,12,14].

In peripheral blood film, the schizont of *P. falciparum* and the gametocytes of *P. vivax* were not seen because it's very rare.

The study tried to give a comparison between the available stages of the erythrocytic cycle of *P. falciparum* and *P. vivax*. The work was conducted with a view to getting as best result as can be, but there is little bit of differences with previous reports. This was due to the calibration of the microscope and due to stain effect.

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References

1. Cattani J, D Davidson and H Engers 1991-92. Malaria. *In:* Tropical Disease Research 11th Programme Report, WHO/World Bank/UNDP. pp 15-17.
2. WHO (World Health Organization). Malaria 1982-1997. Weekly Epidemiological Record 1999 **74**: 265-270.
3. World Health Organization 1997. Malaria in the South-East Asia Region. WHO, Regional Office for South-east Asia, New Delhi. p. 13.
4. Cheng TC 1986. Apicomplexa, Microspora, Ascetospora and Myxozoa. *In:* General Parasitology. Academic Press, Inc. Orlando, Florida. **2**: 824.
5. Haque U, SM Ahmed, S Hossain, M Huda and A Hossain 2009. Malaria Prevalence in Endemic Districts of Bangladesh. PLoS ONE **4**(8): 6737. doi: 10.1371/journal.pone. 0006737.
6. Bangali AM 1996. Border Malaria: Bangladesh Perspective. Journal of Chittagong Medical College Teachers Association. **7**(S3): 95-97.
7. Rahman W, K Chotivanich, K Silamut, N Tanomsing, A Hossain, MA Faiz, AM Dondorp and RJ Maude. 2010. *Plasmodium malariae* in Bangladesh. Trans. R. Soc. Trop. Med. Hyg. **104:** 78-80.

8. Chatterjee KD 1980. Subphylum Sporozoa. *In:* Parasitology (Protozoology and Helminthology). Chatterjee Medical Publishers, Calcutta. **12**: 238.
9. Paul BK 1984. Malaria in Bangladesh. Geographical Review **74**(1): 63-75.
10. Directorate General of Health Services 2005. Current Malaria Situation - Bangladesh. A presentation made for Directorate General of Health Services, GOB, Dhaka. p. 17.
11. Directorate General of Health Services, GOB, Dhaka. http//www.searo.who.int /LinkFiles/ Malaria_in_the_SEAR_Malsitu_endemic_dist_0305.pdf. Visited on 25/03/2010.
12. Gupta BD, RK Maheshwari and M Karunakaran 2006. *Malaria*. BI Publications Pvt. Ltd. New Delhi, India. **2**: 254 pp.
13. Pam DD, NG Imandeh and JA Ajayi 1993. Malaria morbidity among children attending maternal and child welfare clinics in northern Nigeria. Appl. Parasitol. **34**(3): 215-219.
14. Schimdt GD and LS Roberts 2006. Phylum Apicomplexa: Malaria Organisms and Piroplasms. *In:* Foundations of Parasitology. McGraw-Hill, Inc. New York. pp. 702.

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