Original Article

Profile of Mixed Species (*Plasmodium vivax* and *falciparum*) Malaria in Adults

MK Mohapatra*, LK Dash*, PK Bariha, PC Karua*****

Abstract

Objective: Studies on malaria due to co-existent P. falciparum and P. vivax infections are negligible in India. Therefore, this study was undertaken to find out the clinical profile, prognostic factors, and outcome of mixed species malaria and to compare it with P. falciparum malaria.

Methods: This prospective, comparative study has been conducted in a tertiary health care institution with high prevalence of malaria. A cohort of 888 patients of malaria was enrolled in this study. The diagnosis of malaria was made either by Giemsa stained peripheral blood smear or RDT. Mixed species (MS) malaria was diagnosed when both P. vivax and P. falciparum were detected either from peripheral blood smear or RDT. Patients with P. falciparum malaria were grouped in to Pf group. The differences in clinical presentation, biochemical and haematological findings, occurrence of severe malaria, and outcome were recorded, compared, and analyzed. The severity of complication was assessed and Malaria Severity Score (MSS) was calculated. All the patients were treated according to WHO guidelines.

Results: Of them MS and Pf malaria constituted 118 (13.2%) and 770 (86.7%) patients respectively. Severe malaria was found in 17.8% (21 of 118) patients of MS and 57.1% (440 of 770) patients Pf malaria. Pf constituted 440 (95.5%) cases where as MS constituted 21 (4.5%) respectively. The number of severe malaria was significantly ($p<0.001$) more in Pf than MS. Out of 21 cases of severe malaria in MS infection, 14 (66.6%) had single complication and 7 (33.3%) cases had multiple complication. However, in Pf mono infection there were 200 (45.5%) patients with single and 240 (54.5%) with multiple complication. There were 4 independent risk factors for a patient of developing complicated malaria. They were: presenting without fever, high parasite count, Pf mono infection, and fever to treatment interval. Multiple complications and high MSS are associated with increased death in Pf malaria. The outcome of patients of MS was good

Conclusion: In conclusion mixed species infection is not uncommon in the locality where both species coexists. Mixed species infection can complicate with severe malaria. However, its incidence and severity is less than severe falciparum malaria. In mixed infection, P.vivax malaria has a protective effect against the severity of falciparum malaria.

Introduction

Human malaria is caused primarily by 4 different species of Plasmodium namely; P.falciparum (Pf), P.vivax (Pv), P. malariae (Pm), and P. ovale (Po). Clinical pictures, outcome, prognostic factors, and changing clinical pattern of malaria due to individual species infection have been studied extensively.[1] In a geographical area when more than one species coexist, sympatric combination of these infections in an individual can not be ruled out.[2] But profile of malaria due to multiple species infection is considerably underestimated due to lack of studies.[3]

In South-east Asia region, India alone contributes 80% of malaria cases and in India malaria is contributed the most by Orissa state.[4,5] Both Pf and Pv malaria are common in this part of India. However, research on malaria due to co-existent infection of both Pf and Pv is uncommon. Out of few available clinical studies on coincident infection, some studies showed that Pv has a protective effect against severe disease of Pf.[6] On the contrary some studies showed that dual Pf and Pv infection in children increases the disease severity.[7,8] Experimental dual infection of Pf and Pv as a part of malariotherapy in patients with neurosyphilis and mathematical model of parasitic dynamics of Pf and Pv co-infection showed that Pv infection suppresses the severity of Pf.[9,10] There is also no comparative study that describes the differences between mixed and single species malaria in adults. In this context, the knowledge about mixed species infection is important not only for control measures but also for therapeutic options and futuristic vaccine programme. Therefore, we have undertaken this research to study the clinical pattern and outcome of mixed species malaria in adults and to compare it with Pf malaria in a tertiary care hospital.

*Professor, Medicine, **Asst. Professor Medicine, ***Associate Professor Medicine, V.S.S. Medical College and Hospital, Burla, Orissa.
Received: 09.08.2011; Accepted: 24.08.2011

Materials and Methods

This study has been undertaken in the Department of Medicine of V.S.S. Medical College, Burla, Orissa, as a part of our ongoing non-funded prospective observational study on malaria. The vivax arm and falciparum arms of the study had already been reported earlier.[11-13] The present one is the mixed species malaria arm of the above study, in which data from March 2007 to February 2009 are included. To assess the differences in clinical profile and outcome, the data of patients of mixed malaria have been compared with the data of Pf malaria admitted during the period.

All the patients of malaria who were admitted to the indoor had been screened for species diagnosis. Depending on the

Table 1 : Age and Sex Distribution

Age	P. falciparum			Mixed Species		
	Male	Female	Total	Male	Female	Total
18-20	40	20	60	12	4	16
21-30	120	60	180	15	6	21
31-40	140	85	225	22	10	32
41-50	100	30	130	15	5	20
51-60	90	15	105	13	8	21
>61	60	10	70	6	2	8
Total	530	220	770	83	35	118

species all the patients were grouped in to mixed species (MS), Pf, and Pv malaria. Patients of Pv malaria were excluded from the study as the data of Pv malaria had been published earlier.[11] As the intention of the diagnosis of malaria whether single or mixed species infection was to treat therefore the diagnosis was made either with detection of asexual forms of Pf and Pv from Giemsa stained peripheral blood smear, and or rapid diagnostic test (RDT) at the time of admission. However, the parasitic count had been made from the peripheral blood smear only. Parasite counts were expressed as numbers of asexual parasites per micro liter of blood and were calculated from the numbers of parasitized cells per 200 leukocytes in a thick film stained with Giemsa stain i.e. No. of parasites X total leukocyte count / 200. This procedure was followed for each species. Gametocyte counts are made from thick films.

On admission, clinical work up has been done in accordance with the proforma designed for this study. It includes history of previous attack of malaria, antimalarials consumed within four weeks, slide positive report, the area from which hailed, and clinical examination. The laboratory investigations done were : complete blood count (CBC), blood glucose, blood urea, serum creatinine, serum bilirubin, alanine-amino transferase (ALT), aspartate-amino transferase (AST), alkaline phosphatase and glucose-6-phosphate dehydrogenase (G-6-PD), serum sodium, and potassium. Severe malaria was diagnosed according to the guidelines of World Health Organization.[14]

To identify prognostic variables and assess the severity of disease, earlier we did a longitudinal analysis, which enabled us to find out a scoring system known as Malaria Severity Score (MSS).[12] So we calculated MSS taking different variables and analyze it for the outcome. Temperature was recorded and PBS was examined 12 hourly to determine fever resolution and parasitic clearance time. Lumbar puncture was done to study cerebrospinal fluid (CSF) in unconscious patient.

All patients were treated with artesunate as per WHO guidelines.[15] Uncomplicated mixed malaria was treated with artesunate and mefloquine combination and radical treatment was given with primaquin for 14 days. Patients were examined and assessed twice daily until full recovery. All patients were followed up for 1 month after discharge either at the out door or by correspondence.

Analyses were performed with the use of SPSS software, version 10. We analyzed the proportions of sever malaria cases by species. Continuous data are presented as mean ± SD if data were normally distributed. Unpaired data from two groups were compared by two sample t test. Distribution of categorical variables were compared with X^2 test and presented as absolute counts and percentage. A two-tailed probability value of <0.05 was considered statistically significant. CI was calculated at the 95% level. Univariate analysis was done for risk analysis.

Table 2 : Types of severe malaria.

Severe malaria	Falciparum malaria n=440	Mixed malaria n=21	p Value
A. Single Complication			
1. Cerebral malaria (CM)	138(31.3%)	4(19.1%)	<0.01
2. Jaundice (J)	32(7.3%)	2(9.5%)	0.12
3. Anemia (A)	30 (6.8%)	8(38.1%)	<0.001
B. Multiple Complications			
1. CM+A	42 (9.5%)	4(19.1%)	<0.01
2. CM+J	38(8.6%)	1(4.8%)	<0.05
3. CM+J+Renal failure(R)	128(29.1%)	2(9.5%)	<0.001
4. CM+J+A+R	32(7.3%)	0	<0.001
Total number	440 (57.1%)	21(17.8%)	<0.001
Comparison made using X^2 test			

Results

888 diagnosed patients of malaria were enrolled in this study. Of them MS and Pf malaria constituted 118 (13.2%) and 770 (86.7%) patients respectively. Among the patients of MS, there were 83 (70.3%) males and 35(29.6%) females with a ratio of 2.4:1. It is comparable with Pf mono infection. In all the group majority of patients belonged to 21 to 40 years of age group (Table 1).

Severe malaria was found in 17.8% (21 of 118) patients of MS and 57.1% (440 of 770) patients Pf malaria. During the period 51.9% (461 of 888) cases of severe malaria were admitted. Of them Pf constituted 440 (95.5%) cases where as MS constituted 21 (4.5%) respectively. The number of severe malaria was significantly (p<0.001) more in Pf than MS. Out of 21 cases of severe malaria in MS infection, 14 (66.6%) had single complication and 7 (33.3%) had multiple complications. However, in Pf mono infection there were 200 (45.5%) patients with single and 240 (54.5%) with multiple complication. Single complication was more common than multiple complication in MS where as in Pf malaria multiple complication was more common than single complication (p<0.01).The constellation of cerebral malaria, jaundice, and renal failure was the most common complication in severe falciparum malaria (Table 2). The mean malaria severity score (MSS) of MS malaria was 4.7 ± 2.6 where as it was less than Pf mono infection 14.3±2.8 (p<0.001).

Fever was the most common clinical presentation of MS malaria. Intermittent fever with typical paroxysm was present in 110 (93.2%) cases whereas continuous fever was present in 8 (6.8%) cases (Table 3). The intermittent fever was tertian and quotidian in 90 (81.8%) and 10 (9.1%) cases. Ten (9.1%) patients had 2 peaks of typical paroxysm within 24 hours. In Pf malaria fever was present in 552 (71.7%) cases of which intermittent fever was present in 450 (81.5%) cases and continuous fever in 102 (18.4%) cases. In addition to fever complaints like head ache, vomiting, abdominal pain were found in 45 (38.1%) and 400 (51.9%) cases of MS and Pf malaria respectively. 218 (28.3%) patients of Pf mono infection presented with non-febrile complaints only (without fever). Organ specific complaints were present in 440 (57.1%) patients of Pf mono infection which is more than MS malaria (p<0.001). Of them CNS involvement was present in 378 (49.1%) cases. Cerebellar ataxia was found in 8 (2.3%) cases and convulsion was present in 110 (14.3%)

Table 3 : Clinical Presentation

Symptoms and Sign	Falciparum malaria (n=770)	Mixed malaria (n=118)	p Value
I. General Symptoms			
A. Fever	552 (71.7%)	110 (93.2%)	<0.01
1. Intermittent	450 (81.5%)	110 (93.2%)	<0.01
a. Tertian	398 (88.4%)	90(81.8%)	0.14
b. Quotidian	52 (11.5%)	10(9.1%)	<0.01
c. Twice a day	0	10(9.1%)	<0.01
2. Continuous	102(18.4%)	8(6.8%)	
B. Non-febrile Symptoms (along with fever)			
1. Head ache	400(51.9%)	45(38.1%)	<0.01
2. Vomiting/Nausea	350(45.5%)	35(29.6%)	<0.001
3. Abd. Pain	150 (19.5%)	10(8.4%)	<0.05
4. Myalgia	175 (22.7%)	38(32.2%)	0.15
5. Prostration	130 (16.9%)	15(12.7%)	0.1
6. Fainting attack	60(7.8%)	1(0.8%)	<0.05
7. Sleep disturbance	25(3.4%)	2(1.7%)	<0.01
8. Sub conjuctival haemo.	30(3.9%)	1(0.8%)	
9. Non-febrile Symptoms (without fever)	218(28.3%)	0	<0.001
II. Organ Specific			
A. CNS			
1. Unconsciousness	378 (49.1%)	11(1.4%)	<0.001
2. Convulsion	110(14.3%)	1(0.8%)	<0.001
3. Hemiplegia	3 (0.4%)	0	<0.001
4. Paraplegia	2 (0.2%)	0	<0.001
5. Quadruparesis	1 (0.1%)	0	<0.001
6. Retinal haemorrhage	5(0.6%)	0	<0.001
7. Cerebellar ataxia	8(1.1%)	0	<0.001
B. Renal			
1. Oliguria	160 (20.8%)	2(1.68%)	<0.001
2. Black urine	45(5.8%)	0	<0.001
C. Gastro intestinal			
1. Diarrhea	25(3.2%)	0	<0.001
2. Jaundice	230(29.9%)	5(4.2%)	<0.001
3. Abd. Pain	15 (1.9%)	1(0.8%)	<0.01
4. Hepatosplenomegaly	550(71.4%)	110(93.2%)	<0.05
D. Haematological			
1. Bleeding	10(1.3%)	0	<0.001
2. Anaemia	104(13.5%)	12(10.2%)	<0.05

Comparison made using X^2 test

cases Pf malaria. Retinal hemorrhage was present in 5 (0.6%) cases. Splenomegaly with or without hepatomegaly was found in 93.2% and 71.4% cases of MS and Pf malaria respectively. Subconjuctival hemorrhage was found only in 30 (3.9%) cases of Pf malaria.

The diagnosis of malaria was made from peripheral blood smear and RDT test. Out of 118 cases of MS malaria 94 (79.6%) were diagnosed from PBS and rest 24 (20.4%) were diagnosed by RDT test. 550 (71.4%) were diagnosed as Pf malaria from PBS and rest 220 (22.6%) were diagnosed by RDT. The mean parasitic count was 4300.7 ± 180.5/µL in MS and 8756.3 ± 256.3/µL in Pf malaria.

Of 888 patients on admission 34 (3.8%) had gametocytemia. Gametocyte was detected in 8 (6.7%) and 26 (3. 4 %) cases of MS and Pf malaria respectively. The mean gametocyte count was 55.5 ± 15.5/µL in MS and 70.8 ±13.9/µL in Pf malaria (Table 4).

The mean interval of onset of fever to hospitalization is 3.2 ± 1.3 and 5.8 ± 2.8 days in MS and Pf malaria respectively. Patients of MS used to seek treatment approximately 2 times earlier than Pf malaria ($p<0.05$).

Irrespective of species there were 4 independent risk factors for a patient of developing complicated malaria. They were 1) presenting without fever (OR=1.9[95%CI 1.2-3.1], p=0.002), 2) High parasite count >5000/uL (OR=1.8 [95%CI=1.1-2.8],p=0.01), 3) Pf mono infection (OR=4.4[95%CI=1.4-14.3],p=0.02), 4) Fever to treatment interval OR=3.2 [95%CI=2.4-7.3],p=0.002). In MS malaria parasitic count more than 3000/uL (OR=1.6 [95%CI=1.1-2.2],p=0.01) and fever to treatment interval OR=2.8 [95%CI=2.2-5.3],p=0.001) are two risk factors for development of severe disease.

The death was higher in patients with multiple (Vs. single) complication (Univariate HR, 10.7 [CI=3.5-32.8], $p < 0.001$) and high MSS (more than 10) at study enrollment (Univariate HR, 10.2 [CI=4.1-21.0],p=0.002). The outcome of patients of MS was

Table 4 : Base line Characteristics of the study Population

Characteristics	Falciparum malaria (n=770)	Mixed malaria (n=118)	p Value
Male *{n (%)}	550 (71.4)	83 (70.3%)	0.88
Age (Median /Years)	28	26	0.77
Severe malaria* {n(%)}	440(57.1)	21(17.8)	<0.001
Mal Severity Score	14.3±2.8	4.7±2.6	<0.001
Admission Interval (Days)	5.8±2.8	3.2±1.3	<0.05
Parasitic Count (no./ μL)	8756.3±256.3	4300.7±180.5	<0.001
Gametocyte Count (no./ μL)	70.8 ±13.9	55.5 ± 15.5	<0.01
Temp. (°F)	101.0±3.8	104.6±2.8	0.28
Pulse rate (no/mt.)	117.4±13.7	110.2±10.5	0.11
Mean BP (mm Hg.)	103.4±8.9	107.8±3.5	0.40
Resp.rate (no./mt)	27.5±6.5	25.3±5.2	0.36
GCS	6.5 ±2.2	10.9±5.2	<0.05
Hb.(gm/dl)	8.4±2.2	7.8±3.5	0.82
TLC(10^9/L)	9.8 ±2.4	8.6±1.2	0.12
Platelet (10^9/L)	120.8 ± 80.5	180.7±50.9	0.01
B. glucose (gm/dl)	87.4±32.5	90.5±12.5	0.14
S. Sodium (mEq/L)	116.8±8.5	122.6±4.5	0.21
S. potassium (mEq/L)	3.1±1.1	4.1±1.2	0.16
S. bilirubin (mg/dl)	11.9±2.8	3.1±2.7	<0.001
SGOT (IU/L)	61.8±12.2	45.8±10.2	<0.01
SGPT (IU/L)	63.8±12.8	55.8±9.8	<0.01
Alk. Phosphatase (IU/L)	278.9±28.2	125.9 ±19.2	<0.01
Urine output (ml/24 hrs.)	908.8±86.2	1200.8±89.7	<0.01
B. urea (mg/dl)	86.4±24.9	25.2±12.8	<0.001
S. creatinine (mg/dl)	6.2±1.4	1.8±0.3	<0.001

*Comparison made using X^2 test; Rest characteristics: mean (standard deviation), comparison made using t test.

good. Only 1 (0.8%) patient with multiple complications died where as in Pf malaria 70 (9.1%) patients died (p<0.001).

The MS malaria was found more in rainy season i.e. from June to September. In Pf malaria it is perennial with more incidences in the rainy season (Figure 1). During this period total no of severe malaria were also more than dry seasons.

Discussion

Since little information is available on profile of mixed species malaria in India, the present hospital based study is of interest. The most notable finding of the study is that MS can cause severe malaria but its incidence is 3 times less than Pf malaria. The outcome of severe MS malaria is good.

The present study showed that MS malaria was found in 13.2% cases of total hospitalized patients of malaria and of them 17.8% cases had severe malaria. The incidence of MS malaria is variable in different studies. Population survey reported that mixed infection constituted less than 2% of total malaria infection.[6] However, therapeutic studies in Thailand demonstrated that without further exposure 30% of patients with Pf malaria had suffered from symptomatic Pv malaria[16]. Similarly, 8% of patients treated for Pv malaria had cryptic coincident Pf infection that had been diagnosed by PfHRP-2 antigen.[17] It is notable that a high incidence of cryptic mixed infection has been detected by

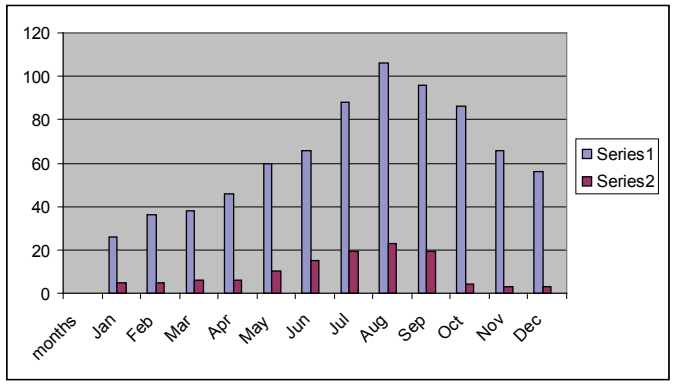

Fig. 1 : Month wise distribution of malaria. (Series-1: Pf and Series-2: MS malaria)

sensitive PCR technique. In a population study where all the 4 species were present, the prevalence of double species infection with PCR was as high as 36.4% and triple infection was 23.7%.[17] Since PCR method is costly and not yet accepted as a routine investigation for diagnosis and treatment of malaria, we used RDT in addition to slide test for the diagnosis of mixed malaria. The available studies have used only the slide test for the diagnosis of malaria.[17,18] The present study had 13.2% cases of MS malaria which is in agreement with other hospital based study (12.7%).[7] The present study showed that the incidence of mixed infection is less than would be expected based on the prevalence of the individual species. Underreporting may play a part, but probably this may be a real phenomenon due to partial cross immunity to heterologous species or biological interference.[3,9]

Geographic heterogeneity and seasonal variation influence the prevalence of MS malaria.[2,3] The present study showed that mixed malaria infections occurred more in wet season than dry season. This seasonal variation may be due to relative abundance of the species in a geographical area. This could result from variations in the presence of mosquito species, which may have species-specific transmission. Seasonal variation of Pf infection with Po and Pv had been reported from Malawi and Papua New Guiena.[8,19] It has been observed that same anopheles mosquito can harbor both Pf and Pv infection and bite of that particular mosquito can inoculate both the species simultaneously.[9] Malaria patients attending during the wet season were more likely to develop severe malaria than dry season. Thus the incidence of MS has been influenced by geographical heterogeneity, seasonal variation, type of study, and diagnostic methods used.

The present study showed that the clinical picture were different from falciparum malaria. Fever was present in majority of cases with typical paroxysm. However, in Pf monoinfection nonfebrile complaints like head ache, abdominal pain and organ specific complaints are more common than MS infection. The organ specific complaints are more because severe malaria was frequently encountered in Pf malaria.

In the present study severe malaria was found in MS malaria but it is 3 times less than Pf mono infection (17.8% Vs 57.1%, p<0.001). However, out of all cases of severe malaria 95.5% cases were due to Pf and 4.5% were due to MS malaria. Thus severe malaria was higher in Pf malaria than MS malaria. Febrile complaints were common in MS malaria where as non-febrile complaints were common in falciparum malaria. The present study is in agreement with other studies that showed a beneficial effect mixed species infection. The coincident infection of Pf and Pv reduces the risk of severe malaria due to Pf by 4 fold.[2,6,9] The

protective potential of Pv against Pf is so much that Pv has been considered as the best available falciparum malaria vaccine. It has also been postulated that α thalasemia are positively selected in a population predisposed to Pv infection and thereby protects against Pf.[6,9] It is notable that in mixed P. malariae and P. falciparum malaria infection also P. malariae protects the severity of falciparum malaria.[20]

All forms of severe malaria like cerebral malaria, anaemia, renal failure, jaundice, multi organ failure had been encountered in adult patients with MS malaria. Earlier hospital based study showed that severe anaemia was the common form of severe malaria in MS infection.[10] In a study from Papua New Guinea, among the children below 5 years, the frequency of severe malaria in dual Pf and Pv infection is 17% which was more than Pf (12%), and Pv (9%) monoinfection. Of them neurological manifestation, anaemia, respiratory distress was found in 8.0%, 5.3%, and 10.3% respectively.[19] The increased risk of severe malaria in children has been attributed to higher overall parasite burden.

Experimental studies in humans and rodents as well as mathematical models revealed a complex parasitic dynamics of MS infection that has clinical consequences affecting the mortality and morbidity.[9,10] One may get severe malaria in mixed species infection depending on whether it is Pf or Pv superinfection. Pv superinfection over an existing Pf infection leads to rise of Pf parasitaemia. It is important since Pv can reappear in the blood following either a new inoculation or a relapse from liver hypnozoites. In this situation, Pv may trigger high Pf parasitaemia that may cause severe malaria. In contrast to the deleterious effect of Pv superinfection, Pf superinfection over an existing Pv infection reduces Pf parasitaemia significantly (by 28%) thus preventing the development of severe malaria. It is notable that simultaneous inoculation by a single mosquito behaves as a Pv superinfection. Simultaneous inoculation will delay the appearance of Pv in the blood by 0.75 days because the pre-erythrocytic stage of Pf lasts for 5.5 to 7.5 days and of Pv for 6 to 8 days.[9] Hence, Pf will appear first and simultaneous inoculation behaves as Pv superinfection causing a rise in Pf parasitaemia.

The following explanations may be put forward for less severe malaria in mixed infection. Firstly, patients with mixed infection developed fever earlier than Pf mono infection. It resulted in early medical attention and treatment reducing the chance of development of severe disease. Clinically the mean duration of seeking treatment in mixed infection was 3.2±1.3 days that is earlier than Pf monoinfection 5.8±2.8 days ($p<0.05$). It is due to low pyrogenic threshold of Pv. The pyrogenic threshold of Pv is 150-200 parasites / μL which is much lower than that of Pf (1500-2000 / μL). Even under conditions of Pv super infection in which Pf grows more rapidly than Pv, the later usually reached its pyrogenic density first seeking the medical attention quickly.[9] Secondly, the presence of interspecies cross immunity prevents from severe malaria.[21] Thirdly, non-immune mechanism probably causes mutual suppression of other species. There may be competition for nutrients in the blood stream.[21]

In conclusion mixed species infection is not uncommon in the locality where both species coexists. MS infection can complicate with severe malaria but its incidence is significantly less suggesting a protective effect of Pv infection. It is important because, if previous Pv infection does protect against severe Pf infection, then control of vivax malaria may reduce the vivax-associated protection against severe falciparum infection enhancing increased incidence of severe falciparum malaria.

References

1. White NJ. Malaria, In Manson's Tropical Diseases, 22nd ed. Eds. Cook GC, Zumla AI, Elsevier, 2009, 1201-1300.
2. McKenzie FE and Bossert WH. Mixed species Plasmodium infections of humans. *J Parasitol* 1997;83:593-600.
3. Mayxay M, Pukrittayakamee S, Newton PN, White NJ. Mixed species malaria infections in humans, *Trends in Parasitol* 2004;20:233-240.
4. WHO. World Health Report 2008.Geneva: *World Health Organization* 2008.
5. Mohapatra MK. Current status of drug-resistance malaria in India, *Medicine Update*, API, Eds. Agarwal AK, 2009;1:9-20.
6. Price R, Nosten F, Simpson JF, Luxemburger C, et al. Risk factors for gametocyte carriage in uncomplicated falciparum malaria. *Am J Trop Med Hyg* 1999;60:1019-1023.
7. Gopinathan VP, Subramanian AR. Vivax and falciparum malaria seen at an Indian service hospital. *J Trop Med Hyg* 1986;89:51-55.
8. Bruce MC, Macheso A, Kelly-Hope LA, Nkhoma S, etal. Effect of transmission setting and mixed species infections on clinical measures of malaria in Malawi, *PLos ONE*, 2008, 3(7):e2775. doi:10.1371/journal.pone.0002775.
9. Mason DP and McKenzie FE. Blood stage dynamics and clinical implications of mixed Plasmodium vivax and Plasmodium falciparum infections, *Am J Trop Med Hyg* 1999;61:367-374.
10. Boyd MF, Kitchen SF. Vernal vivax activity in persons simultaneously inoculated with Plasmodium vivax and Plasmodium falciparum. *Am J Trop Med* 1938;18:505-514.
11. Mohapatra MK, Padhiary KN, Mishra DP, Sethy G. Atypical manifestations of Plasmodium vivax malaria. *Ind J Malario* 2002;39:18-25.
12. Mohapatra MK & Das SP. The malaria severity score: a method for severity assessment and risk prediction of hospital mortality for falciparum malaria in adults. *J Asso Phys Ind* 2009;57:119-126.
13. Mohaptra, M.K. The natural history of complicated falciparum malaria-a prospective study. *J Asso Phys Ind* 2006;54:848-53.
14. World Health Organization. Severe falciparum malaria. *Trans Roy Soc Trop Med Hyg* 2000;94:(Supl.1).1-90.
15. Guidelines for the treatment of malaria. *World Health Organization* 2006;1-182.
16. Luxemburger C, Ricci F, Nosten F, Raimond D, etal. The epidemiology of severe malaria in an area of low transmission in Thailand. *Trans R Soc Trop Med Hyg* 1997;91:256-262.
17. Mayxay M, Pukrittayakamee S, Chotivanich K, Imwong M, etal. Identification of cryptic coinfection with Plasmodium falciparum in patients presenting with vivax malaria. *Am J Trop Med Hyg* 2001;65:588-592.
18. Mehlotra RK, Lorry K, Kastens W, Miller SM, et al. Random distribution of mixed species malaria infections in Papua New Guinea. *Am J Trop Med Hyg* 2000;62:225-231.
19. Genton B, D'Acremont V, Rare L, Baea K, et al. Plasmodium vivax and mixed infctions are associated with severe malaria in children: A prospective cohort study from Papua New Guinea. *PLoS Med* 2008;5:e127.doi:10.1371/journal.pmed.0050127.
20. Mason DP, McKenzie FE, Bossert WH. The blood stage dynamics of mixed Plasmodium malariae- Plasmodium falciparum infections, *J Theor Biol* 1999;198:549-566.
21. Maitland K, Williams TN, Newbold CI. Plasmodium vivax and P. falciparum: biological interactions and the possibility of cross-species immunity. *Parasitol Today* 1997;13:227-231.