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Ahaptoglobinemia (HpO) and Malaria in India

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Haptoglobin (Hp) polymorphism analysed among *P. vivax* and *P. falciparum* patients and malaria negative subjects from areas with different epidemiological situations had shown high incidence of ahaptoglobinemia (HpO) among malaria patients. A definite association of HpO with *P. vivax* as well as *P. falciparum* malaria in Indian subjects had been observed. However, low sensitivity and reliability of HpO index indicates that it can not be a good indicator for determination of malaria endemicity. About 75 per cent of HpO subjects with *P. vivax* infection when treated with chloroquine showed typable Hp polymorphs by 8–9 days of post-treatment.

Keywords: Antimalarials, HpO, *P. falciparum*, *P. vivax*

INTRODUCTION

Ahaptoglobinemia or hypohaptoglobinemia (HpO) is represented by low or nondetectable levels of haptoglobin (Hp), an alpha-2-glycoprotein having haemoglobin binding property. This is generally a temporary phenomenon caused by masking of genetically determined phenotypes of haptoglobin. Three genetic variants of haptoglobin (Hp), namely Hp 1-1, 2-1 and 2-2 have been described by Smithies[1]; and Smithies and Walkar.[2] These variants are controlled by two autosomal alleles Hp^1 and Hp^2. However, genetic ahaptoglobinemia (true Hp^0 allele) due to absence of functional gene and no gene product has also been reported by Harris *et al.*[3]

In African population strong evidences have been indicated that malaria and functional

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ahaptoglobinemia are closely related.[4–11] Hill *et al.*[12] had also supported the involvement of malaria in producing acquired ahaptoglobinemia in Melanesian populations which are genetically distinct from Africans.

As in the African continent, malaria is still a major public health problem in India and about 2.5 to 3.0 million malaria positive cases are detected every year (Source: NAMP). Preliminary studies carried out on haptoglobin polymorphism have shown increased prevalence of functional ahapto-globinemia among malaria positive subjects compared to malaria negative subjects.[13–15] Therefore, study was extended with the following objectives: (i) to study haptoglobin polymorphism, prevalence of ahaptoglobinemia and association with malaria, if any among Indian groups; and (ii) to know at what time period after the antimalarial treatment, haptoglobin polymorphs are typable in malaria patients with nontypable haptoglobin phenotypes (HpO or ahaptoglobinemia).

MATERIALS AND METHODS

Samples were analyzed from different regions of the country with varying malaria epidemiology. Data collected during present and earlier studies were tested for the association of HpO with *Plasmodium falciparum* and *P. vivax* positivity and validity of HpO for the diagnosis of malaria positivity.

Study areas

Labour camp (LB), Delhi: Delhi, the capital of India, is situated at 28° 30' latitude north and 77° 12' east longitude and *P. vivax* is the predominant species of malaria parasite in the area.[16] In Delhi due to various developmental and construction activities, a floating population of migrant labourers is present which come from different parts of the country and contribute greatly to the malaria situation. Study area is mostly comprised of temporary hutments and inhabited by tribals from different parts of the country. The camp was located in southern part of Delhi, and had a localized outbreak of falciparum malaria during the study period—post-monsoon months of 1992.

Gurgaon district (Mewat region), Haryana: Surveys were conducted during April 1997 in two CHCs namely Nuh and Ferozepur Zhirka of Mewat region of District Gurgaon (approximately 27°N and 70°E). Mewat region of District Gurgaon had experienced an outbreak/epidemic of malaria during post-monsoon season in 1996. Four villages each of CHCs Nuh and Ferozepur Zhirka were surveyed. This region is mostly Muslim dominated with few Hindu villages or families. In general, people belong to lower economic strata. Both *P. falciparum* and *P. vivax* were prevalent in this area. During the survey, proportion of *P. falciparum* cases was about 36.3 per cent.

District Mandla, Madhya Pradesh: Samples were collected from villages of District Mandla (approximately 22° 50' latitude and 80° 59' longitude) during 1988 by conducting spot fever surveys. Mandla is a *P. falciparum* endemic area situated in tribal belt with rocky and undulating terrain with thick forest. Most of the villages are located near the streams. Tribals con-

stitute 80 per cent of the population and a focus of chloroquine resistance has also been reported in the area.[17]

Malaria Clinic, MRC, Delhi: Blood samples were collected during 1993 from patients visiting the Malaria Clinic of Malaria Research Centre, Delhi, located in the northern zone of Delhi. People residing in nearby residential colonies and peri-urban areas of northern region comprise a good proportion of the patients. Being a metropolis, population is comprised of a mixture of various ethnic, social and economic groups. Blood samples were collected from patients before and after antimalarial therapy.

Methodology

Blood (100–200 µl) was collected in heparinized vials (1.5 µl microfuge) by finger prick method from the subjects reporting to Malaria Clinic or by conducting mass blood surveys after obtaining verbal consent. A smear was also made simultaneously for parasite examination. This study has clearance of the ethical committee of the Centre.

Blood samples were transported to the laboratory in an ice box within 2–3 hours of collection. Samples were centrifuged and the plasma was removed into another tube and stored at –20°C till use. Details of methodology for haptoglobin analysis and parasite identification are given in our earlier studies.[13,18]

Fisher's chi-square test was applied to test the level of significance and Wolfe's chi-square test as used by Bayoumi *et al.*[19] was adopted to study the association of polymorphs with malaria. Data collected in this study and from our earlier reports[13,15] were further tested for specificity, sensitivity and predictive value of the HpO marker to signify *P. falciparum* and *P. vivax* positivity in the population and to estimate the efficiency of HpO for malaria diagnosis.[20] Association was measured by testing null hypothesis (Ho)—no association, phi (ϕ) statistics and heterogeneity χ^2- analysis.[21]

RESULTS

Haptoglobin polymorphism and ahaptoglobinemia

In the present study all three common phenotypes of Hp: Hp 1-1, 2-1 and 2-2 were observed. Figure gives the distribution of Hp polymorphs, calculated allelic frequencies and proportion of HpO in study areas. Allelic frequencies calculated for Hp^1 ranged from 0.1 to 0.23 and 0.77 to 0.90 for Hp^2 in the study areas. Allelic frequencies were calculated after deleting HpO samples. Distribution of the polymorphs in each area followed the Hardy-Weinberg equilibrium (chi-square values were nonsignificant). Percentage of samples showing nontypable Hp polymorph (HpO or functional ahaptoglobinemia) varied from 20.7–52.82. Fisher's comparative chi-square analysis of malaria positive and malaria negative groups revealed that distribution of polymorphs was significantly different between the two groups in all the study areas (chi-square values were between 14.72 and 20.83; $p < 0.001$). Chi-square values ranging between 10.28 and116.28 by Wolfe's method have fur-

ther confirmed the significant difference. A significantly higher incidence of HpO was observed among the malaria patients in the study areas. It is also observed that proportion of Hp 2-2 has decreased significantly among malaria patients compared to malaria negative subjects (Fig. 1).

From the figure it is further revealed that significantly higher incidence of HpO among the control samples (malaria negative) in areas with malaria outbreak—general population of labour camp, Delhi (HpO 32 per cent) and in villages of Mewat region of District Gurgaon, Haryana (HpO 23 per cent), compared to malaria endemic region of Mandla, M.P. (HpO 9.6 per cent). Comparative chi-square values were 7.61 and 7.68 ($p < 0.01$) for LB Delhi versus Mandla and Mewat region versus Mandla respectively.

Among the samples studied from LB population, Delhi, *P. falciparum* was the prevalent

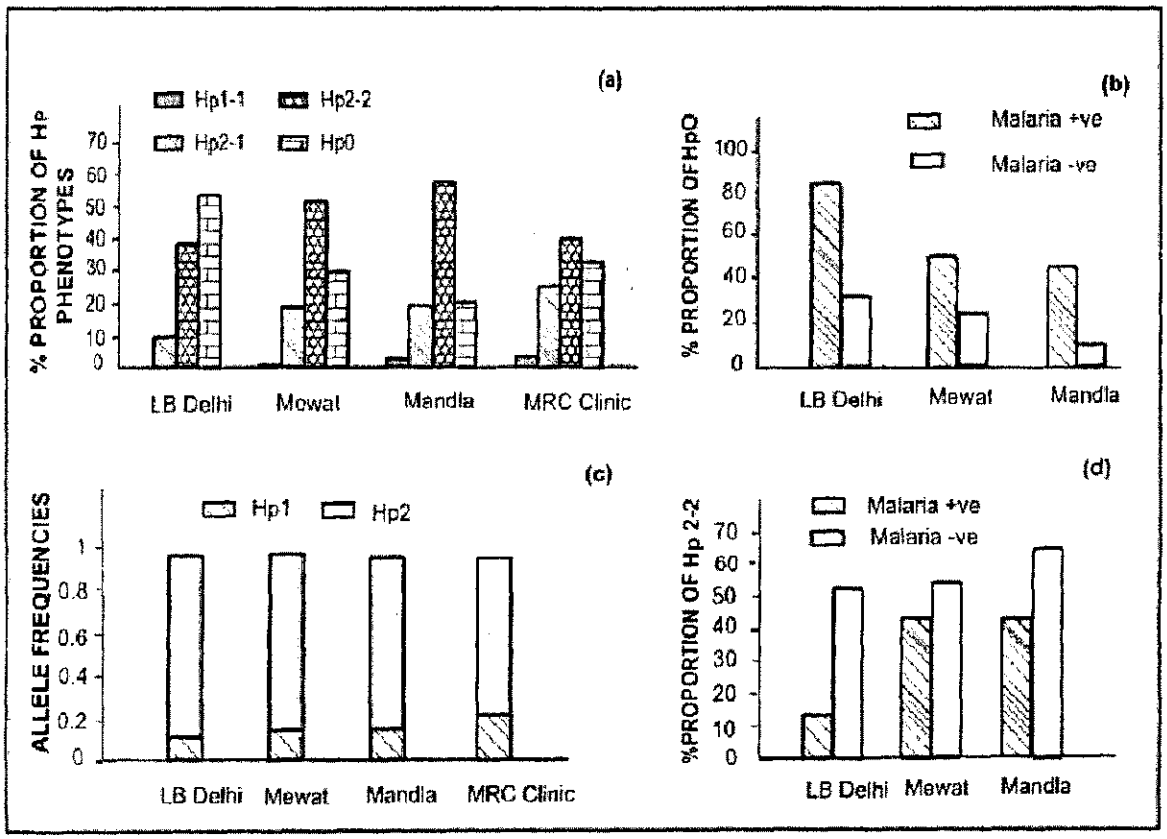

Fig. 1: Distribution of Hp polymorphs: (a) proportions of Hp polymorphs in different study areas; (b) HpO proportions among malaria positive and malaria negative subjects; (c) allelic frequencies in study areas; and (d) proportion of Hp 2-2 among malaria positive and malaria negative subjects in the study area

species (85.7 per cent) and the proportion of nontypable samples was significantly higher in *P. falciparum* positive subjects (83.3 per cent) than the negative controls.

In Mewat region of District Gurgaon which has experienced malaria epidemic during post-monsoon months of 1996, both *P. falciparum* (36.4 per cent) and *P. vivax* (63.6 per cent) were prevalent. Proportion of HpO among the general population was about 29.1 per cent with much higher proportion of HpO about 50 per cent among the malaria positive subjects and 64.7 per cent among the *P. falciparum* patients (11/18).

In District Mandla (M.P.), HpO incidence was about 20.7 per cent in general population and among *P. falciparum* malaria positive group, it was observed to be 44.7 per cent.

HpO and association with malaria

Analysis of data from five population groups for *P. falciparum* and three groups for *P. vivax* have shown that chi-square values were significant in all the groups except one in *P. vivax* (Table 1a–b). In Buksa population, limited number of *P. vivax* cases (33) were detected during the study and all were non-HpO which have given nonsignificant chi-square value. Measurement of association of HpO with *P. falciparum* malaria has given ϕ values ranging from 0.2 to 0.65 in five areas, while for *P. vivax* 0.08 to 0.22 in three areas. Also heterogeneity chi-square analysis was found to be significant ($p < 0.05$) for heterogeneity factor for *P. falciparum* study areas and for *P. vivax* study areas it is nonsignificant.

HpO : Malaria diagnostic efficiency

Table 2 gives sensitivity, specificity and predictive values for positive and negative results of HpO for *P. falciparum* and *P. vivax* diagnosis in different areas. Efficiency of the HpO for *P. falciparum* diagnosis in different areas was between 68.8 and 81.1 per cent with cumulative efficiency of 74.7 per cent while that for *P. vivax* was between 61.1 and 84.8 per cent with cumulative value of 71.6 per cent.

HpO after antimalarial therapy

In a total of 107 *P. vivax* patients analyzed for haptoglobin polymorphism during July to November 1993 from Malaria Clinic of MRC, Delhi, 35 patients were found to be of HpO giving a percentage of 32.7 (Fig. 1). Out of these 35 patients, 19 could be followed-up for Hp polymorphs after antimalarial therapy. Drug dosage was given as per NAMP policy.

It is observed from Table 3 that by Days 8–9 of post-chloroquine therapy, most of the subjects (75 per cent) tested showed typable haptoglobin levels. However, in some of the subjects typable Hp forms could not be observed till Day 13. Subjects could not be followed further. As the subjects of the study were *P. vivax* positive patients, therefore, in addition to chloroquine, radical treatment with antirelapse drugs was also given. Study was coincided with clinical trials of bulaquine, a new compound developed by CDRI, India, as antirelapse drug, therefore, further analysis was done under three different groups—subjects who received primaquine (standard antirelapse drug being used for *P.*

Table 1(a). Null hypothesis : Chi-square analysis

Area	*Pf* (+)		*Pv* (+)		Non-Mal		*Pf* vs Normal		*Pv* vs Normal	
	HpO	Non-HpO	HpO	Non-HpO	HpO	Non-HpO	χ^2	ϕ	χ^2	ϕ
Delhi										
Malaria Clinic, MRC	19	52	35	169	10	247	37.3	0.34	22.8	0.22
Labour Camp	20	4	–	–	8	17	13.3	0.52	–	–
Haryana										
Distt. Gurgaon (Mewat Region)	11	7	17	21	56	177	11.75	0.21	7.2	0.16
Madhya Pradesh										
Distt. Mandla (Gond tribe)	17	15	–	–	7	76	27.7	0.49	–	–
Uttar Pradesh										
Haldwani, Distt. Nainital (Buksa tribe)	29	132	0	33	23	312	14.4	0.20	2.5	0.08
Total	96	210	52	223	104	829	69.7	0.24	12.7	0.10

Table 1(b). Heterogeneity χ^2 analysis

	χ^2	N	Deviation	Variance ratio
P. falciparum				
Deviation	69.7	1		
Heterogeneity	34.8	3	11.6	Significant at 0.05 probability point
Total	104.5	4		
P. vivax				
Deviation	12.7	1		
Heterogeneity	19.8	1	19.8	Nonsignificant
Total	32.5	2		

N = Degrees of freedom.

Table 2. Sensitivity, specificity and predictive power of HpO for the malaria diagnosis

Area (Population)	Sensitivity	Specificity	Predictive value		Efficiency	Reliability
			For (+) results	For (–) results		
			P. falciparum			
Delhi						
Malaria Clinic, MRC	26.8 (16.4–37.3)	96.1 (93.7–98.5)	65.5 (47.9–83.1)	82.6 (78.6–87.0)	81.1 (76.7–85.5)	0.23 (0.18–0.28)
Labour Camp	83.3 (68.1–98.5)	68.0 (49.4–86.6)	71.4 (54.4–88.4)	80.95 (69.2–92.8)	75.5 (63.5–87.5)	0.51 (0.37–0.65)
Haryana						
Mewat Region	61.1 (38.0–84.1)	76.0 (70.4–81.6)	16.1 (7.1–25.1)	96.2 (93.4–99.0)	74.9 (69.5–80.3)	0.37 (0.31–0.43)
Madhya Pradesh						
Mandla (Gond tribe)	53.1 (35.5–70.7)	91.6 (85.6–97.6)	70.8 (52.2–89.4)	79.8 (71.4–88.2)	80.9 (73.5–88.3)	0.45 (0.36–0.54)
Uttar Pradesh						
Haldwani (Buksa tribe)	18.1 (12.0–24.0)	93.1 (90.2–95.8)	55.8 (42.0–69.6)	70.3 (65.9–74.7)	68.8 (64.6–73.0)	0.11 (0.08–0.14)
Cumulative	31.4 (26.1–36.7)	88.9 (86.9–90.9)	48.0 (41.0–55.0)	79.8 (77.3–82.3)	74.7 (72.2–77.2)	0.20 (0.18–0.22)
			P. vivax			
Delhi						
Malaria Clinic, MRC	17.2 (12.0–22.4)	96.1 (93.7–98.5)	77.8 (66.6–89.0)	59.4 (54.6–64.2)	61.1 (56.6–65.6)	0.13 (0.10–0.16)
Haryana						
Mewat Region	44.7 (28.5–60.9)	76.0 (70.4–81.6)	23.3 (15.5–35.1)	89.4 (85.0–93.8)	71.6 (66.2–77.0)	0.21 (0.16–0.26)
Uttar Pradesh						
Haldwani (Buksa tribe)	0.0	93.1 (90.3–95.9)	0.0	90.4 (87.2–93.6)	84.8 (82.1–87.5)	0.07 (0.04–0.10)
Cumulative	18.9 (14.1–23.7)	89.2 (87.0–91.4)	36.9 (28.7–45.1)	76.8 (74.0–79.6)	71.6 (68.8–74.4)	0.08 (0.06–0.10)

Figures in parentheses are confidence interval at 95 per cent level.

vivax), bulaquine and placebo. In primaquine group, five out of eight HpO subjects tested showed typable Hp pattern within 16 days while in bulaquine group, three out of six HpO subjects showed typable Hp pattern within eight days. In placebo group, 75 per cent (3 out of 4) subjects showed typable pattern within 12 days.

Table 3. Data on ahaptoglobinemia status of *P. vivax* positive subjects after chemotherapy

Days after infection with chloroquine therapy	No. of HpO samples tested	No. of samples showing typable Hp phenotypes
6	1	1 (2-2)
8	8	6 (2-1, 2-2)
9	3	2 (2-2)
11	2	0
12	2	1 (2-2)
13	2	1 (2-2)
16	1	1 (2-2)

Hp phenotypes are given in parentheses.

All the patients who showed typable Hp polymorphs on Day 0 remained so, later on, except one patient who showed Hp 1-1 on Day 0 became Hp 2-1 after 15 days. Majority—81.82 per cent of the ahaptoglobinemic patients became Hp 2-2 in follow-up study, while only two (18.2 per cent) became Hp 2-1. None was of Hp 1-1 type.

DISCUSSION

Indian populations are generally characterized by high Hp^2 gene frequencies.[22] Observed allele frequencies of Hp^1 (0.1 to 0.23) and Hp^2 (0.77 to 0.90) in the present study samples from different areas fall within the reported range for the other Indian populations. However, the incidence of genetic ahaptoglobinemia has been reported to be low, in the range of zero to seven per cent.[18, 22–24] Observed significantly high incidence of ahaptoglobinemia among *P. falciparum* and *P. vivax* malaria positive subjects compared to malaria negative group in the present study (Fig. 1) proposes the role of malaria as a causative agent for functional ahaptoglobinemia in Indian subjects.

Reversal of HpO level to typable Hp phenotypes after antimalarial treatment of ahaptoglobinemic malaria patients (*P. vivax*) also presents supporting evidence to above findings. An earlier study carried out among malaria susceptible and refractory subjects, showed those with frequent attacks of malaria (three or more) had a higher frequency of HpO (42.2 per cent) than those who did not have any (29.6 per cent),[14] suggesting the role of malaria in the depletion of haptoglobin levels.

No association between *P. falciparum* and *P. vivax* cases and HpO suggest that hypothesis is not tenable, thus the association of HpO with *P. falciparum* and *P. vivax* malaria is considered (χ^2 values are presented in Table 1b). Association of HpO with *P. falciparum* and *P. vivax* has shown wide range of ϕ values (0.2 to 0.65) between five different areas of *P. falciparum* and 0.08 to 0.22 between three different areas of *P. vivax*. Thus, it can be concluded that association of HpO with *P. falciparum* as well as *P. vivax* malaria is present in the Indian population.

Low sensitivity and reliability (Table 2) of HpO indicated that HpO can't be used as an index to study malaria positivity in the Indian population. On the other hand, specificity of about 90 per cent for *P. falciparum* and *P. vivax* in total samples suggest absence of HpO among population speculates absence of disease (malaria) in the population with 90 per cent accuracy.

Correlation of HpO with *P. falciparum* malaria had been reported in the African population by many workers.[4–11,25] Rougemont *et al.*[10] reported that HpO can be used as a malariometric index in hyper-endemic areas at least equal to the specificity of the clinical indicators but not with malaria parasite index. In contrary, Boreham *et al.*,[8] had shown significant correlation of HpO with malariometric indices, parasitaemia and splenomegaly. Sisay *et al.*,[26] on the other hand suggested use of serum Hp levels as an indicator of the efficacy of malaria intervention trials. The observation of Monjour *et al.*[25] supports our observation by ruling out the use of haptoglobin levels as a diagnostic test for present or past malarial infection in upper volta population. Reports on the use of HpO index as malariometric index are not conclusive. Meta-analysis of the data from various studies can help in giving a concrete conclusion.

Faster recovery in our subjects unlike slow recovery observed in African subjects of Hp levels after chemotherapy with antimalarials in ahaptoglobinemic patients may be because: (i) patients included in the study were of *P. vivax* which attains less severity than *P. falciparum* and is a predominant species of human malaria parasite in India (Delhi); and (ii) quicker diagnosis and treatment: patients were residing mostly in Delhi city or peri-urban areas and were aware of the malaria clinic in their locality, and come for diagnosis within 2–3 days of illness, thus immunity levels and severity of the disease were low in the patients. Thus, epidemiological situation may be playing a vital role in the recovery of Hp levels in the ahaptoglobinemic patients.

Comparative delayed recovery of Hp levels in ahaptoglobinemic patients after antirelapse drug therapy compared to placebo may be due to their toxic effect on RBC which are known to be prone to hemolysis after intake of certain antirelapse drug—primaquine.[27] Hemolytic effects are less in case of bulaquine and, therefore, justify the better recovery in case of bulaquine than primaquine treated group.[28] These are preliminary observations, and an in-depth study on this aspect will be helpful to develop concrete hypothesis.

Study by Elagib *et al.*[29] have shown an association of Hp 1-1 phenotype with *P. falciparum* malaria among Sudanese population. They report a high incidence/proportion of Hp 1-1 (common phenotype of Sudanese) among the malaria positive subjects (both complicated and non-complicated). Surprisingly, no case of HpO was observed by them.

Similarly, Quaye *et al.*[30] in a study conducted in Accra, Ghana (Africa) has observed an association of Hp 1-1 phenotype with susceptibility to *P. falciparum* malaria and to severe disease. In their study area Hp 1-1 was the predominant phenotype that is reported to have high Hb-binding efficiency. In contrast in Indian population (present study) Hp 2-2 is the predominant phenotype which is reported to have low Hb-binding efficiency thus becoming non-detectable (HpO) levels caused by utilization of large amount of Hp 2-2 in formation of Hp-Hb complex during malaria infection. This may explain their observation of no significant difference in the HpO incidence between malaria positive and negative control groups in Ghana population. This

can also explain the Hp phenotype of a subject changing from Hp 1-1 to Hp 2-1 on Day 15 of post-treatment in the present study—Hp^2 products were nontypable on Day 0 but became typable on Day 15. Hill *et al.*[12] have also reported that Hp 2-2 and Hp 2-1 phenotypes have decreased Hb-binding efficiency compared to Hp 1-1 phenotype.

This is the first study carried out on a large-scale in Indian population showing a definite association of malaria with ahaptoglobinemia, more so this is the only study showing association of ahaptoglobinemia with *P. vivax* malaria, which is predominant human malaria parasite species in southeast Asia, Latin America and Papua New Guinea.

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Evaluation of Repellent Action of Neem Oil against the Filarial Vector, *Culex quinquefasciatus* (Diptera: Culicidae)

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Studies were carried out to evaluate the repellent action of neem oil against *Culex quinquefasciatus*. Application of 2 and 5 per cent neem oil @ 5 ml/person/night gave 50 and 40.9 per cent protection in indoor collections and 17.4 and 5.6 per cent in outdoor collections as compared with that of untreated control respectively. The protection time ranged from 0100 to 0300 hrs and 0100 to 0600 hrs in indoor and outdoor collections respectively. Results of repellent action of Autan, a synthetic mosquito repellent studied concurrently showed a relatively higher protection rate from the bites of *Cx. quinquefasciatus*.

Keywords: *Culex quinquefasciatus*, Mosquito repellent, Neem

INTRODUCTION

Azadirachta indica, the common neem tree is traditionally known for its medicinal value.[1] On account of its antifeedant and insecticidal properties, neem has been widely used in agriculture particularly in pest control.[2,3] In recent years, the use of neem against vectors of human diseases has been greatly considered. The effect of neem derivatives on mosquitoes has been intensively studied.[4–7] Neem oil has been recognized as a candidate repellent for personal protection by topical application on exposed skin. In the present paper, we report the results on the efficacy of neem oil (2 and 5 per cent) as a repellent against *Culex quinquefasciatus*, the vector of bancroftian filariasis.

MATERIALS AND METHODS

The study was carried out in two adjacent residential colonies, Jayanthi and Ganga-Cauvery colony of Anna Nagar, Chennai from August 1995 to January 1996. All night human bait

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mosquito collections were adopted. A total of 10 trials, five trials each with 2 and 5 per cent neem oil were undertaken. During every trial, a total of four collections, each on neem oil (either 2 or 5 per cent), coconut oil (Tata brand), autan (15 per cent—DEET, Bayer India Ltd.) and untreated control were conducted concurrently in indoors and outdoors. Neem oil, marketed by Unjha Ayurvedic Pharmacy, Gujarat was used in the present study. Prior to collections, the required concentration of neem oil was prepared using coconut oil. A total of 5 ml of the test repellents were thoroughly applied to all the exposed parts of the baits (volunteers) like hands, legs, neck and face. The volunteers were allowed to lie down and all mosquitoes landing on the exposed parts were collected with the help of an aspirator. Hourly collections were kept in separate containers and were brought to the laboratory for identification. In order to ensure uniformity and to avoid bias, the collectors, baits and catching stations were shuffled on a rotational basis. A standard Orthogonal Greco Latin Square method was used for the purpose.[8] Evaluation was undertaken against an untreated control. The repellent effect of coconut oil and autan was assessed for the purpose of comparison. Percentage protection was calculated using the following formula:

$$\% \text{ protection} = \frac{\text{No. of mosquitoes (Control} - \text{Experimental)}}{\text{No. of mosquitoes collected in control}} \times 100$$

RESULTS

A total of 80 human bait collections were undertaken during the study. The temperature recorded indoors and outdoors during the collections ranged from 19 to 33°C and 18 to 32°C respectively and humidity recorded ranged between 42 and 94 per cent during indoor collections and 52 and 98 per cent in outdoor collections. Both *Anopheles* and *Culex* species were obtained during the collections. Among anophelines, *An. stephensi* and *An. subpictus* were obtained. *Cx. quinquefasciatus*, the common pestiferous mosquito and vector of bancroftian filariasis was the only major culex species observed. Maximum number of *Cx. quinquefasciatus* were obtained during the third quarter of the night (1200 to 0300 hrs) in both indoor and outdoor collections. Table 1 summarizes the results of the study.

The results show that application of 2 per cent neem oil gave 50 and 17.4 per cent protection against *Cx. quinquefasciatus* in indoor and out-

Table 1. Repellent action of neem oil against *Culex quinquefasciatus* on human volunteers

Neem oil	Trial	Total	Mean (range)
		Indoor	
	Con	2538	507.6 (109–1319)
	Exp	1268	253.6 (56–651)
2%		*Outdoor*	
	Con	1284	256.8 (146–557)
	Exp	1057	211.4 (73–366)
		Indoor	
	Con	1823	364.6 (137–500)
	Exp	1078	215.6 (59–340)
5%		*Outdoor*	
	Con	1165	233.0 (42–413)
	Exp	1110	220.0 (50–470)

Con—Control; Exp—Experimental.

door collections respectively. Hourly collections showed consistent protection in indoor only. The percentage protection recorded during the peak biting quarter were 38.69 per cent (1200 to 0100 hrs), 45.8 per cent (0100 to 0200 hrs) and 65.57 per cent (0200 to 0300 hrs) in indoor collections. Protection time varied between 0100 and 0300 hrs in both indoor and outdoor collections. Application of 5 per cent neem oil gave 40.9 per cent protection in indoor and 5.6 per cent in outdoor. During the peak biting quarter the percentage protection obtained in indoor were 33.33 per cent (1200 to 0100 hrs), 38.78 per cent (0100 to 0200 hrs) and 21.13 per cent (0200 to 0300 hrs). The protection time varied from 0100 to 0300 hrs in indoor and 0100 to 0600 hrs in outdoor.

The average man-biting rate per night in trials with coconut oil and autan during 2 per cent neem oil trial was 152.2 ± 125.6 and 51.8 ± 36.8 in indoor and 220.4 ± 179.6 and 34.0 ± 29.9 in outdoor respectively. During trials with 5 per cent neem oil, it was 98.8 ± 24.5 and 36.2 ± 19.9 in indoor and 164.4 ± 104.9 and 90.8 ± 145.3 in outdoor respectively. The relative protection obtained in indoor and outdoor collections on application of neem oil, coconut oil and autan is shown in Figs. 1 and 2. The protection time in coconut oil varied from 0100 to 0200 hrs and 0100 to 0300 hrs in indoor and outdoor collections respectively. In autan, protection time of 0400 to 0900 hrs and 0400 to 0800 hrs were obtained in indoor and outdoor.

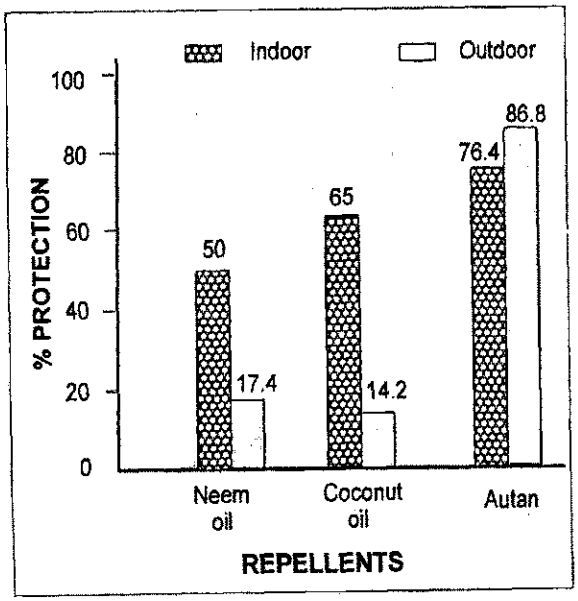

Fig. 1: Relative protection from mosquito bites per night observed in indoor and outdoor collections during 2 per cent neem oil trial

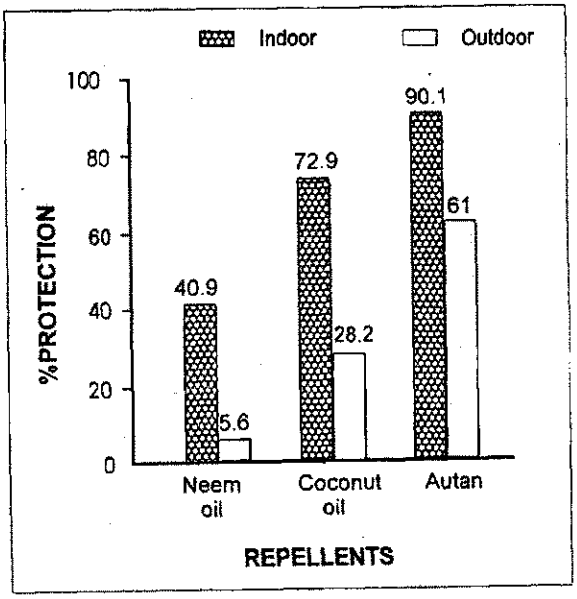

Fig. 2: Relative protection from mosquito bites per night in indoor and outdoor collections during 5 per cent neem oil trial

DISCUSSION

In the present study, the efficacy of neem oil as a repellent against *Cx. quinquefasciatus* was evaluated. The results revealed man mosquito contact to occur continually from dusk-to-dawn. Maximum man landing rate was observed in indoor. Neem oil offered protection from the bites of *Cx. quinquefasciatus*. Comparatively, more protection was observed indoors. Nevertheless, the total protection obtained indoors did not exceed 50 per cent. The percentage protection obtained was less when compared with the results of other studies. Kant and Bhatt[9] reported more than 75 per cent protection with 2 per cent neem oil in indoor. In outdoor collections Sharma *et al.*[10] obtained 61 per cent protection. The variation in protection can be attributed to factors like absorption, abrasion or evaporation of the compound that directly effects the persistency and influences its efficacy. Coconut oil used as a base provided greater protection than neem oil and this may be the reason for increased protection in 2 per cent neem oil which requires further investigation. The protection time was lowest in coconut oil. Autan, the synthetic repellent exhibited maximum repellency with prolonged protection time.

From the study, it is evident that neem oil provided moderate protection from the bites of *Cx. quinquefasciatus*. The continuous man mosquito contact exhibited by this species and the minimal protection time provided by neem oil may limit the use of neem oil as an effective repellent. However, the tolerability of different strains of the species to neem oil is to be assessed before drawing any valid conclusion. Development of neem formulations that provide prolonged duration of protection with social acceptability is essential. Use of neem oil with other compounds to enhance the repellency, needs to be further investigated.

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Atypical Manifestations of *Plasmodium vivax* Malaria

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About 110 patients were enrolled to study the atypical presentations and the chloroquine sensitivity pattern of *Plasmodium vivax* malaria. The diagnosis was made from Giemsa stained peripheral blood smear. The co-infection of falciparum malaria was excluded both by smear and ParaSight F-test. After a thorough clinical work up, biochemical investigations were done. The fever clearance and parasite clearance time were determined in all cases. Absence of malarial paroxysm (22.8 per cent), migrainous headache (4.5 per cent), myalgia (6.3 per cent), episodic urticarial rash (1.8 per cent), relative bradycardia (13.6 per cent) and postural hypotension (2.7 per cent) were the atypical manifestations encountered. Besides this, severe forms like jaundice (7.2 per cent), cerebral involvement (0.9 per cent), severe anaemia (7.2 per cent), thrombocytopenia (3.6 per cent) and pancytopenia (0.9 per cent) had been detected. All, except the patient with cerebral involvement were treated with chloroquine patients responded well to the treatment except two (1.8 per cent) patients who had chloroquine resistance. This study showed that vivax malaria can present with atypical and protean manifestations. The changing clinical profile along with development of chloroquine resistance may be considered as a warning signal.

Keywords: Chloroquine resistance, Protean manifestations, Vivax malaria

INTRODUCTION

Plasmodium vivax malaria is the second commonest cause of malaria in the globe.[1] Unlike falciparum malaria, severe forms of vivax malaria are uncommon. Severe vivax malaria has been described in Europe in the past, which was possibly related to malnutrition and other inter-

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current disease.[1] Recently due to the development of resistance to chloroquine, a change in the clinico-epidemiological profile of vivax malaria has been apprehended.[2] Hence, it is not unlikely to get vivax malaria with unusual manifestation which may be life threatening. Therefore, the present prospective study has been undertaken to study the atypical manifestations of vivax malaria and its therapeutic response to chloroquine.

MATERIALS AND METHODS

Study site and plan

The present study has been conducted at Mohandas Karam Chand Gandhi (M.K.C.G.) Medical college hospital, Berhampur, Orissa. The state Orissa is endemic for malaria and contributes to $^1/3^{rd}$ of total malaria patients and half of total malaria deaths in the country.[3] This hospital is the tertiary care centre of the southern part of Orissa, which is the major contributor of malaria patients in the state. Therefore, we have launched a non-funded prospective observational study on malaria since 1995. The present one is the vivax arm of the above study, in which data from March 1998 to February 2000 are included.

Patient selection

A total of 110 patients of vivax malaria who were admitted during the period to the indoor or treated at the outdoor, were selected for this study. The diagnosis has been made with detection of the parasite (*P. vivax*) in the peripheral blood smear (PBS) by Giemsa stain. The parasitic count was determined by the formula (No. of parasites x total leukocyte count)/200. Co-infection of *P. falciparum* was ruled out when no parasite was detected on repeated PBS examination and a negative test for histidine rich protein II (HRP-II) antigen of *P. falciparum* in the blood by ParaSight F-test (Becton Dickinson, USA). Patients with mixed infection, history of renal and liver disease, alcohol addiction were excluded from the study.

Methods

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 : haemoglobin (Hb), defferential and total leukocyte count (TLC), platelet count, 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). ParaSight F-test was done in all cases to exclude associated falciparum infection. Temperature was recorded and PBS was examined 12 hourly to determine fever resolution and parasite clearance time. Lumbar puncture was done to study cerebrospinal fluid (CSF) in unconscious patient.

The patients were treated with oral chloroquine 600 mg base at 0 and 24 hours followed by 300 mg at 48 hours. One case of vivax malaria with cerebral involvement was treated with parenteral quinine followed by oral quinine.

Chloroquine resistant cases were treated with oral quinine for seven days. For radical treatment, primaquine, 15 mg daily for 14 days had been administered to the patients with normal G-6-PD level and 45 mg weekly for eight weeks to G-6-PD deficient individuals.[4] After treatment all the patients were followed-up at monthly interval for one year at the outdoor or by correspondence.

Statistical analysis and graphs were done by MS-Excel. Comparison of means were performed by Student's 't' test and p-value less than 0.05 was considered significant.

RESULTS

Out of 110 patients, there were 70 males and 40 females. The age ranged from 15 to 60 years. Majority were between 31 and 40 years (Table 1). Of 110 patients, 60 (54.5 per cent) had history of fever for last two months. Of them 25 (41.6 per cent) had received antimalarial (chloroquine) inadequately. Only 11 (10 per cent) patients had a positive report for vivax malaria.

Table 1. Age and sexwise malaria distribution

Age (yrs)	Male	Female	Total
15–20	9	5	14
21–30	17	8	25
31–40	27	12	39
41–50	12	10	22
51–60	5	5	10
Total	70	40	110

The clinical presentation of malaria was presented in Table 2. Intermittent fever with typical paroxysm was present in 85 (77.2 per cent) cases whereas continuous fever was present in 10 (9.1 per cent) cases. Fever was absent in 15 (13.6 per cent) cases at the time of presentation. Frontal headache, migrainous headache and generalised myalgia were found in 25 (22.7 per cent), 5 (4.5 per cent) and 7 (6.3 per cent) cases respectively. Splenomegaly could be detected in 90 cases (81.8 per cent) whereas hepatosplenomegaly was found in 25 (22.7 per cent) cases. Episodic urticarial rash was recorded in 2 (1.8 per cent) patients. Relative

Table 2. Clinical presentation

Clinical presentation	No. of cases	Percentage
Fever		
(a) Intermittent	85	77.2
(b) Continuous	10	9.1
(c) No fever	15	13.6
Headache		
(a) Frontal headache	25	22.7
(b) Migrainous	5	4.5
Generalised myalgia	7	6.3
Urticarial rash	2	1.8
Jaundice	8	7.2
Diarrhoea	1	0.9
Neurological	1	0.9
Haematological		
(a) Severe anaemia	6	2.5
(b) Thrombocytopenia	4	3.6
(c) Pancytopenia	1	0.9
Cardiovascular		
(a) Relative bradycardia	15	13.6
(b) Postural hypotension	3	2.7

Table 3. Abnormal liver function tests in vivax malaria

Parameter	Abnormal (n = 8)	Normal (n = 102)	p-value
S. bilirubin (mg/dl)	3.25 ± 1.5	0.9 ± 0.02	< 0.05
AST (IU/l)	28.8 ± 6.3	25.5 ± 5.2	N.S.
ALT (IU/l)	30.0 ± 3.5	26.5 ± 2.1	N.S.
Alkaline Phosphatase (KA Units)	10.2 ± 3.5	8.4 ± 1.2	N.S.

N.S.— Nonsignificant.

bradycardia was found in 15 patients (13.6 per cent). Postural hypotension was observed in three patients (2.7 per cent).

Eight (7.2 per cent) patients presented with jaundice. The mean onset of jaundice was 5.5 ± 2.5 days. Serum bilirubin, AST, ALT, and alkaline phosphatase were 3.25 ± 1.5 mg/dl, 28.8 ± 6.3 IU/l, 30 ± 3.5 IU/l and 10.2 ± 3.5 KA units respectively (Table 3). All patients with jaundice had hepatosplenomegaly. Serum bilirubin returned to normal within 5 to 10 days (6.5 ± 3.2 days) after initiation of treatment. One (0.9 per cent) patient presented with acute gastroenteritis.

Neurological involvement in the form of unconsciousness (cerebral vivax malaria) was found in a female patient who had delivered one month ago. Glasgow coma scale of the patient was five. The hematological profile of the patient was: Hb–8 g/dl, TLC–8200/m^3, platelet count 1.2 lakhs/m^3, and differential count was within normal limits. The biochemical investigations were random blood glucose—90 mg/dl, blood urea — 32 mg/dl, s. creatinine—0.8 mg/dl; serum Na^+ — 130 mEq/l; serum potassium — 3.5 mEq/l. CSF analysis was within normal limits. The patient became conscious after 48 h of quinine therapy.

Severe anaemia (Hb < 6 g/dl) was found in 8 (7.2 per cent) patients, with mean Hb, 4.2±1.6 g/dl. Moderately severe anaemia (Hb: 6–9 g/dl, mean = 8.2±1.9 g/dl) was present in 60 (54.5 per cent) patients. Rest 42 (38.1 per cent) patients had mean Hb of 10.1±2.1g/dl. Thrombocytopenia (platelet count < 1,00,000) was detected in 4 (3.6 per cent) patients, mean count was 75,000 ± 225.2/m^3. One patient had peripheral pancytopenia with normal bone marrow picture. G-6-PD deficiency was found in 4 (3.6 per cent) patients.

All the patients except the patient with cerebral involvement were treated with chloroquine. The fever resolution and parasite clearance time had been depicted in Fig. 1. The mean fever resolution time was 38.2 ± 10.2 hours (8 to 50 h) and parasite clearance time was 36.8 ± 8.5 hours (12 to 56 h). It had been observed that 2 (1.8 per cent) patients were chloroquine resistant. (Fig. 2). The patient having R-II resistance was a 30 year old male who had fever one month ago for which he had taken chloroquine adequately. The second patient having R-III resis-

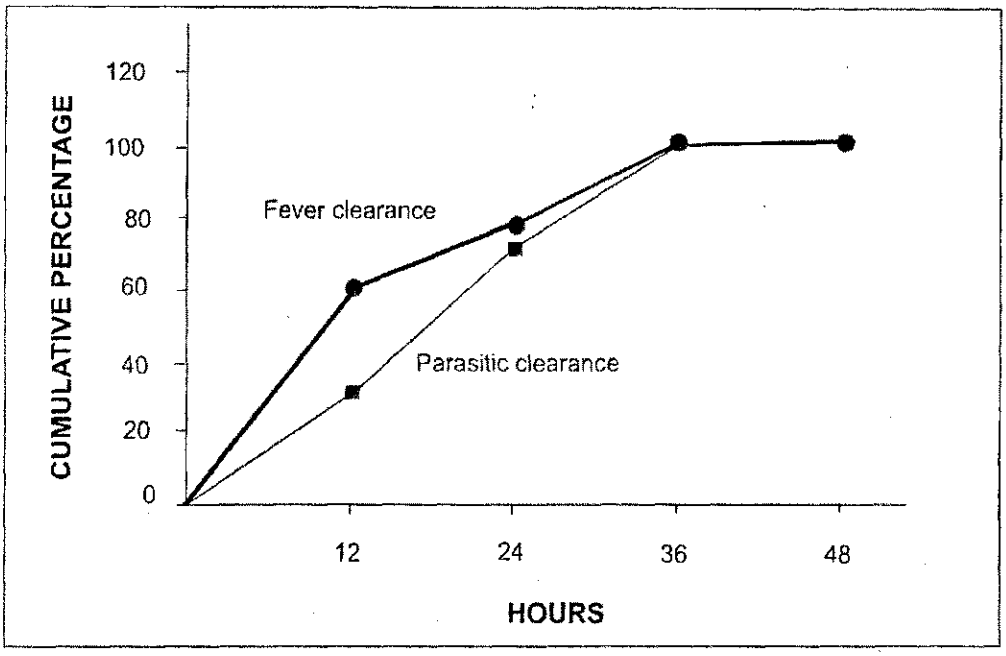

Fig. 1: Cumulative percentage of fever and parasitic clearance

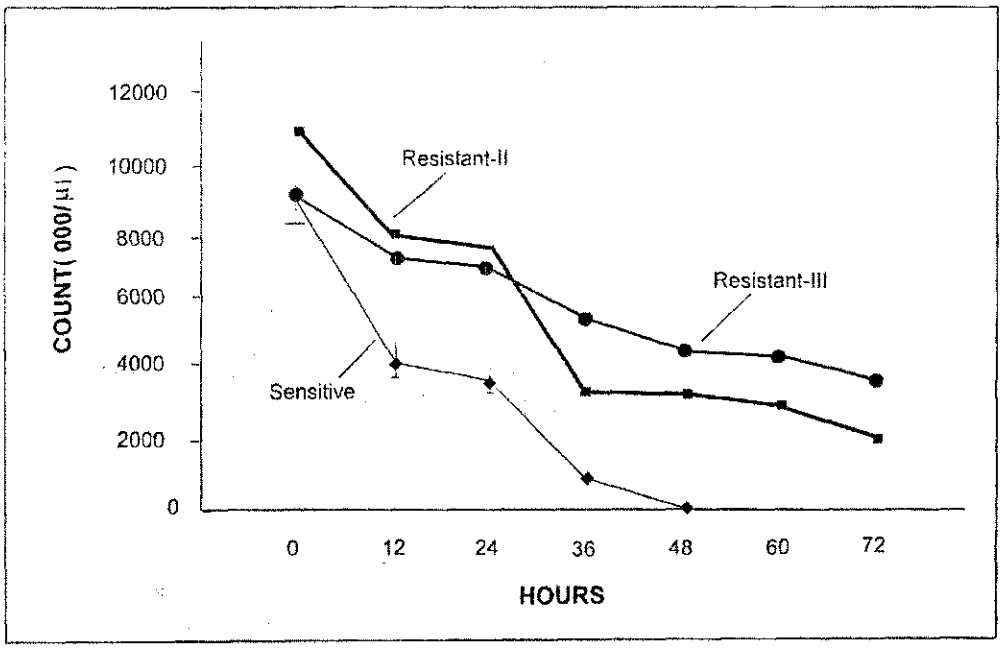

Fig. 2: Parasitic count in chloroquine sensitive (S) and resistant (R-II and R-III) vivax malaria

tance was a 40 year female without any history of similar attack previously and also history of taking any antimalarial. In both patients neither urine nor blood level of chloroquine was estimated. All patients recovered completely with treatment.

DISCUSSION

Studies on vivax malaria are limited because of the benign nature of the disease. But the present research revealed not only a changing clinical profile but also an emerging resistance of vivax malaria to chloroquine in this part of the country. Absence of typical malaria paroxysm, migrainous headache, myalgia, episodic urticarial rash, relative bradycardia and postural hypotension were the atypical manifestations encountered in vivax malaria. Absence of fever, presence of pyrexia equivalents like episodic rash, headache are the uncommon observations found in this study. It also showed that 20 (18.2 per cent) cases have protean presentations. Of them jaundice, haematological abnormalities, cutaneous manifestations, gastroenteritis and cerebral involvement was present in 8 (7.2 per cent), 8 (7.2 per cent), 2 (1.8 per cent), 1 (0.9 per cent) and 1 (0.9 per cent) cases respectively. Even if vivax malaria has been considered as a benign disease, there are reports documenting its neurological,[5–8] haematological,[9,10] hepatic,[11] and renal complications.[12] Earlier Upadhyay and Bhalla[13] reported eight cases of vivax malaria with protean manifestations of which jaundice, anaemia and gastroenteritis was present in 4, 2 and 2 patients respectively. Renal failure as a complication of vivax malaria was found only in one case.[12]

Jaundice has been reported at presentation in vivax malaria.[1,11] The present study showed that jaundice, mild to moderate in severity was present in 7.2 per cent cases and the patients recovered completely within a week (5.5 ± 2.5 days). Apart from hyperbilirubinaemia, AST and ALT were raised twice of the normal in those patients. Non specific hepatitis with or without jaundice, can occur in patients with vivax malaria. Liver biopsy in vivax malaria demonstrated brown granules and small granulomatous lesion.[11] One patient presented with diarrhoea in this series.

Central nervous system manifestations in vivax malaria are less common. However, lesser degree of obtundation and delirium has been reported.[1] Cerebral vivax malaria has been documented with *P. vivax* multinucleatus infection in China and with malnutrition in Europe.[1] Two cases of vivax malaria with cerebral involvement have been recorded from India.[6,7] On rare occasions whenever cerebral malaria has been attributed to *P. vivax*, co-infection with *P. falciparum* always appeared as a possibility.[5–7] However, in the present case in addition to negative PBS, negative HRP-II antigen has also been taken into consideration to exclude falciparum co-infection. The case of cerebral vivax malaria in this series was a female who delivered a baby a month ago. Associated malnutrition may be a predisposing factor for cerebral involvement in this case. The patient recovered completely with treatment. Parenteral quinine has been administered in this case because of apprehension of chloroquine resistant vivax malaria in this locality.

Apart from cerebral involvement, hemiplegia,[5] peripheral neuropathy[6] and ataxia[1] have also been described in vivax malaria. In the present series we have not encountered those complications. Neither in this nor in other series, complications like hypoglycaemia and seizures have been encountered.

Haematological complications like severe anaemia, thrombocytopenia and pancytopenia was found in 7.2, 3.6 and 0.9 per cent cases respectively. *P. vivax* has been attributed as the cause of pancytopenia in a patient of chronic myeloid leukaemia after allogenic blood stem cell transfusion.[10] Even without any disease it can induce severe anaemia and thrombocytopenia.[9] Relative bradycardia and postural hypotension have been observed in vivax malaria.

G-6-PD deficiency was detected in 3.6 per cent cases of vivax malaria. None of the patients who had jaundice, severe anaemia and chloroquine resistant were G-6-PD deficient. Chloroquine resistant vivax malaria was found in 2 (1.8 per cent) cases, RII, and RIII one case each. Similar incidence of resistant benign tertian malaria was detected in 1 to 2 per cent cases in Mumbai.[2] After the first report of chloroquine resistant vivax malaria from Papua New Guinea in 1989, subsequent reports form different parts of the world including India has appeared.[14] However, lack of *in vitro* study and estimation of chloroquine in blood are the limitations of most of the reports including the present one.

Different regimens have been advocated for treatment of chloroquine resistant vivax malaria. Those include quinine sulfate 600 mg eight hourly daily with or without doxycycline for seven days, chloroquine (25 mg base/kg) plus primaquine (2.5 mg/kg) in three divided doses over 48 hours, extended course of chloroquine (600 + 600 + 300 + 300 mg base) followed by primaquine 15 mg for 14 days and mefloquine.[14] As an anti-relapse medication in G-6-PD deficient subjects 45 mg of primaquine weekly for eight weeks has been recommended.[4,14]

In spite of these complications, there was no mortality in the present series. The patients responded well to treatment. Except a couple of resistant cases, all became afebrile and aparasitaemic by 48 hours of chloroquine therapy. The outcome of vivax malaria is invariably good. Not a single death had been reported during the epidemic of 1964 in Sri Lanka.[1] Similarly no death had also been reported from 2573 cases of vivax malaria in Thailand.[15] However, a patient of vivax malaria with shock and multi organ involvement died in Czech Republic.[16]

Though pernicious syndromes in vivax malaria has been documented occasionally, inapparent mixed co-infection with *P. falciparum* could not be excluded adequately.[4–6] In none of the reports, HRP-II antigen of *P. falciparum* had been utilised for exclusion of falciparum malaria. As this test does not cross react with other species of malaria like *P. malariae, P. ovale* and *P. vivax*, a negative ParaSight F-test in a patient of vivax malaria almost excludes the possibility of falciparum co-infection.[17] The mechanism of pernicious manifestations of vivax malaria is not clearly understood. Strain variations, associated malnutrition and depressed immunity may contribute for severe manifestations of vivax

malaria, which needs further research. The gradual development of resistance to chloroquine and pernicious manifestations of benign tertian malaria may be considered as a warning signal. Hence, health personnel should take appropriate measures in the treatment of *P. vivax* infection.

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Enhancement of Antimalarial Activity of Chloramphenicol against Indian *Plasmodium falciparum* Isolates *in vitro* by Chloroquine

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The antimalarial activity of chloramphenicol, a broad-spectrum antibiotic was studied in three Indian *Plasmodium falciparum* isolates having high or low sensitivity to chloroquine. The antimalarial activity was evaluated in 72 hours culture in the presence of various doses of chloramphenicol alone or in combination with a fixed dose of chloroquine added at a concentration that is well tolerated by parasites *in vitro*. Significant growth inhibition with chloramphenicol was observed at concentrations above 10 µg/ml in three isolates. However, an increase in the antimalarial activity of the compound at low concentrations in the range of 0.25–2.5 µg/ml has also been observed in the presence of chloroquine. Parasite growth was significantly inhibited in chloroquine resistant parasites. Use of chloramphenicol with chloroquine may be useful as a combination therapy to improve efficacy of the antimalarials and to retard the development of resistance to the individual components of the combination.

Keywords: Chloramphenicol, Chloroquine, Drug sensitivity, *In vitro* culture, Malaria, *P. falciparum*

INTRODUCTION

Chloramphenicol is a broad-spectrum antibiotic, which is obtained from cultures of the soil bacterium, *Streptomyces venezuelae*. It exerts its antimicrobial effects by targeting bacterial ribosome.[1] Antimalarial activity of chloramphenicol was reported about 50 years ago. Trials were conducted with various antibiotics including chloramphenicol in human vivax and falciparum malaria.[2,3] Data, thus generated showed that clearance of parasitaemia and fever was slower

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with the antibiotics than chloroquine and other antimalarials. With these results, use of antibiotics was not considered in the treatment of malaria. Rieckmann *et al.*[4] first demonstrated the use of the antibiotic, tetracycline and its efficacy in chloroquine resistant *P. falciparum* strains. Tetracycline and quinine in combination are being used as effective antimalarials for the treatment of *P. falciparum* infection in those areas where parasites have become resistant to chloroquine and other antimalarials.[5] Now a days combination therapy in malaria especially in resistant infections is getting a high priority.[6] Chloramphenicol as an effective antibiotic, is often given to patients suffering from pyrexia of unknown origin, where malaria is not ruled out. In this situation, this drug may be helpful in combination with a rapidly acting antimalarial in treating malaria infection also.

Present study was undertaken to determine the activity of chloramphenicol alone in chloroquine sensitive and resistant *P. falciparum* strains *in vitro* and also to determine if there was any increase in antimalarial activity of chloramphenicol with a fixed concentration of chloroquine or with various doses of chloroquine with a fixed concentration of chloramphenicol.

MATERIALS AND METHODS

Drug and preparation of culture plates

Chloramphenicol (CPL) in the powdered form was obtained from Boehringer Mannheim GmbH, Germany. A stock solution of 5 mg/ml was prepared in 0.2 ml ethanol (for dissolving) and 0.8 ml sterile 0.015M phosphate buffered saline (PBS), pH 7.2. Wells of flat bottom 96-well tissue culture plates were dosed with 250, 100, 50, 25, 10, 5, 2.5, 1, 0.5 and 0.25 μg/ml concentrations. Chloroquine phosphate (CQ) in powdered form was obtained from SIGMA Chemical Company (St. Louis, MO, USA). A stock solution of 2.56 mg/ml was prepared in PBS. Wells of 96-well tissue culture plates were dosed with 256, 128, 64, 32, 16, 8, 4 and 2 ng/ml concentrations. In CPL dosed plates, CQ at a concentration of 16 ng/ml was added and in plates dosed with CQ, CPL at a concentration of 10 μg/ml was added in each well respectively. Wells with plain PBS (without drug) were kept as control. Drug dosed plates were dried under laminar flow, then sealed and stored until use.

Parasite lines

Indian *P. falciparum* isolates with variable sensitivity to CQ (*in vivo* and *in vitro*) were used in the study. Chloroquine and sulpha-pyrimethamine (SP) sensitive and resistant strains were selected on the basis of patients' response and *in vitro* susceptibility of clinical isolates to the drugs. In brief, the methodology is as follows: Patients reported in local/field clinic with fever were screened for malaria parasite. *P. falciparum* positive cases with asexual parasitaemia of above 1000 parasites/μl blood with no history of taking CQ or other antimalarial drugs; with no history of intolerance to CQ were enrolled for *in vivo* and *in vitro* drug sensitivity tests (Day 0) after obtaining informed consent from the patients. An extended 28-day field test was used to monitor the therapeutic efficacy of both CQ and SP as per WHO guide-

lines and protocol.[7–9] Enrolled patients were administered orally a total dose of CQ—25 mg/kg and a total dose of SP—25/1.25 mg/kg body weight. Thick and thin blood smears were examined for the presence of asexual parasite (if any) on Day 1, 2, 7, 14, 21 and 28 giving a total observation period of four weeks in each case. The *in vitro* test was performed on Day 0 as per the published method.[7]

Three *P. falciparum* isolates with variable sensitivity to antimalarials were used in this study. The FDL-B and FDL-NG isolates were obtained from patients belonging to Delhi; FSH-14 was isolated from patient belonging to Shankargarh, Allahabad. The FDL-B isolate is sensitive to both CQ and SP, while FDL-NG showed RII grade resistant to CQ but sensitive to SP and FSH-14 showed RII grade resistance to both.[10] These parasites were grown, adapted and maintained in continuous culture by candle-jar technique using established protocol.[11]

In vitro assay

The antimalarial activity of CPL, CQ alone or in combination with both was determined in the three parasite lines by setting cultures in four sets of respective drug dosed plates. The inoculum in each well consisted of 200 µl of five per cent erythrocyte suspension having 0.5 per cent synchronized ring form parasite in RPMI-1640 media enriched with 10 per cent human AB serum (v/v) obtained from a healthy normal person. Parasites in the presence of drugs were allowed to grow at 37°C for 72 h. Growth of the parasite from each well was monitored microscopically by counting the number of schizonts per 200 asexual parasites and total number of parasites per 5000 RBCs. Per cent schizont maturation inhibition (SMI) and total parasite growth inhibition (PGI) were calculated by the formula: (1-Nt/Nc) x 100, where Nt and Nc represent the number of parasites in the test and control wells respectively. Minimum inhibitory concentration (MIC)—the concentration of the drug which killed nearly all the parasites, was calculated for determining the effect of CPL/CQ alone and in combination of each other.[12]

Statistical analysis

The differential response of three *P. falciparum* isolates to CPL and CQ and to combinations of both the drugs was determined by the method of one-way analysis of variance. To compare the MICs with single drug or combinations, Spearman rank test was used. The result was considered significant at five per cent level.

RESULTS

Parasites were exposed to various concentrations of CPL alone or in combination with fixed dose of CQ *in vitro* for 72 h. Substantial effect of CPL on schizont maturation and overall parasite growth was achieved above 10 µg/ml concentration (Table 1). However, in the presence of CQ at a concentration of 16 ng/ml (3.2 ng per well) parasite growth and reinvasion were significantly inhibited even at a lower concentration of 0.25 µg/ml in case of CQ-sensitive FDL-B isolate ($p<0.01$) and at 2.5 to 5 µg/ml in case of resistant lines FDL-NG and FSH-14 ($p<0.001$). As observed, there was an en-

Table1. Effect of chloramphenicol alone and in combination with chloroquine in chloroquine sensitive (FDL-B) and resistant (FDL-NG, FSH-14) *P. falciparum* isolates in 72 h culture

CPL (μg/ml)	CPL / CPL+CQ (16 ng/ml)											
	FDL-B				FDL-NG				FSH-14			
	% SMI (CPL)	% SMI (CPL+ CQ)	% PGI (CPL)	% PGI (CPL+ CQ)	% SMI (CPL)	% SMI (CPL+ CQ)	% PGI (CPL)	% PGI (CPL+ CQ)	% SMI (CPL)	% SMI (CPL+ CQ)	% PGI (CPL)	% PGI (CPL+ CQ)
250	100	100	100	100	100	100	100	100	100	100	100	100
100	100	100	100	100	100	100	100	100	100	100	100	100
50	100	100	94	100	100	100	90	100	100	100	90	100
25	80	100	82	100	82	100	69	100	75	100	70	100
10	60	100	70	94	72	100	66	94	67	100	53	90
5	50	100	55	85	45	83	35	79	50	60	37	73
2.5	10	83	24	79	9	67	17	67	17	50	17	63
1.0	0	75	9	79	0	33	7	45	8	30	7	37
0.5	0	75	0	76	0	25	0	39	0	20	0	27
0.25	0	75	0	79	0	33	0	27	0	20	0	23

SMI—Schizont maturation inhibition; PGI—Parasite growth inhibition.

hancement of the effect of CPL along with CQ in parasite growth in all the three isolates (Table 1).

Chloroquine susceptibility (CQS) profile of the three parasite isolates confirmed their sensitivity and resistant status *in vitro*. Parasites growth below 16 ng/ml was considered as sensitive, whereas parasite growth at or above 32 ng/ml was considered as resistant as per published protocol.[13] Inhibition in growth of FDL-B was observed at a low concentration of 4 ng/ml CQ. In two drug resistant lines, parasites growth occurred even at 128 ng/ml CQ concentration (Table 2). Addition of CPL at 10 μg/ml (2 μg per well) caused inhibition in schizont maturation and in total growth of the parasites even at lowest concentration—2 ng/ml (Table 2). Parasiticidal activity of CQ in combination with CPL was increased about four times in FDL-B ($p< 0.05$) and about five to six times in FDL-NG and FSH-14 ($p< 0.001$).

The drug concentrations, which inhibited 90 per cent schizont maturation or total parasite growth were compared. Parasite growth in CPL alone

Table 2. Effect of chloroquine alone and in combination with chloramphenicol in chloroquine sensitive (FDL-B) and resistant (FDL-NG, FSH-14) *P. falciparum* isolates in 72 h culture

CQ concentration (ng/ml)	CQ/ CQ+CPL (10 µg/ml)											
	FDL-B				FDL-NG				FSH-14			
	% SMI (CQ)	% SMI (CQ + CPL)	% PGI (CQ)	% PGI (CQ + CPL)	% SMI (CQ)	% SMI (CQ + CPL)	% PGI (CQ)	% PGI (CQ + CPL)	% SMI (CQ)	% SMI (CQ + CPL)	% PGI (CQ)	% PGI (CQ + CPL)
256	100	100	100	100	100	100	100	100	100	100	93	100
128	100	100	100	100	82	100	69	100	75	100	67	100
64	100	100	100	100	64	100	59	100	50	100	50	100
32	100	100	100	100	36	100	24	100	42	100	40	93
16	70	100	67	100	27	100	21	100	25	100	20	90
8	50	100	45	91	27	100	10	91	8	80	7	80
4	30	82	38	79	9	75	0	67	0	70	3	60
2	10	67	12	67	0	75	0	64	0	60	0	60

SMI—Schizont maturation inhibition; PGI— Parasite growth inhibition.

Table 3. Minimum inhibitory concentration values of chloramphenicol and chloroquine alone and in combination of each other on parasite schizont maturation and total growth

Parasite lines		MIC in µg/ml (Mean±S.D.)		MIC in ng/ml (Mean±S.D.)	
		CPL	CPL in combination with 16 ng/ml CQ	CQ	CQ in combination with 10 µg/ml CPL
FDL-B	% SMI	27±1.02	2.7±0.13	18.5±0.80	4.4±0.62
	% PGI	37±3.05	7.0±0.36	19±0.71	7.7±0.67
FDL-NG	% SMI	26±1.93	5.4±0.83	140±10.98	4.6±0.37
	% PGI	50±1.31	7.80±0.43	145±6.48	7.8±0.88
FSH-14	% SMI	28.5±1.56	6.2±0.96	140±5.69	8.8±0.98
	% PGI	50±1.53	10±0.99	235±5.89	16±1.42

SMI—Schizont maturation inhibition; PGI—Parasite growth inhibition; MIC—Minimum inhibitory concentration.

or in combination with CQ showed significant reduction in MIC ($r = 0.839$; $p < 0.02$). Values of MIC, in case of CQ alone or in combination with CPL showed significant changes ($r = 0.715$; $p < 0.05$) as observed in CPL+CQ combination (Table 3).

DISCUSSION

From the present study it is observed that CPL can affect *P. falciparum* growth *in vitro* efficiently when parasites were exposed at least for two complete cycles of ring form to schizont development. Schizont maturation rate was significantly reduced at 10 μg/ml CPL. In an earlier study, antimalarial activity of CPL was observed after 96 h in established *P. falciparum* lines FC and K-1. Minimum inhibitory concentrations were in the range of 10.7 to 12.5 μg/ml.[14] Parasites, which were grown in CPL+CQ dosed culture, where CQ was added at a borderline concentration, generally caused 50 per cent inhibition in almost all CQS lines but well tolerated by the resistant falciparum lines. Additional dose of CQ caused notable effect in the three parasite isolates. Results were encouraging when parasites were exposed to variable doses of CQ with an additional fixed dose of CPL. Chloroquine resistant parasites showed higher sensitivity as observed from the growth inhibition profile. Findings of the study indicated that addition of CQ or CPL caused a boost in antimalarial activity *in vitro*, this effect could be additive or synergistic.

Generally, *in vitro* results of most of the compounds are very promising but sometimes come out as ineffective in human use due to their pharmacokinetic properties. Antimicrobial agents like doxycycline, tetracycline, clindamycin, minocycline, erythromycin, etc. have been evaluated for their antimalarial activity, but were found to have limited potential and more side effects.[15] Chloramphenicol as a bacteriostatic protein synthesis inhibitor was tested in *P. falciparum* and also in *Babesia rodhaini* and *Theileria parva* culture lines to check its antiprotozoal activity in order to determine its suitability for routine inclusion in protozoan cultures. Among three protozoans, *P. falciparum* showed high sensitivity to CPL *in vitro*.[16,17] In a recent study, effect of CPL on nucleoside-5' triphosphates (NTPs) and 2' -deoxynucleoside-5' triphosphates (dNTPs) levels were compared in *P. falciparum* and human leukaemia cells. The drug caused more severe effects on the levels of NTPs and dNTPs in *P. falciparum* compared to leukaemia cells, which may provide an explanation for its selective toxicity against malaria parasite.[18]

In Malawi, clinical overlap of bacteraemia with malaria and anaemia is common and causes delay in diagnosis. In Malawian children above six months age, non-typhoidal Salmonella bacteraemia was significantly associated with malarial parasitaemia. Antibacterial *in vitro* resistance to ampicillin (79 per cent), cotrimoxazole (72 per cent), gentamicin (55 per cent) was very common in comparison to CPL (0.3 per cent). Thus, chloramphenicol became the antibiotic of choice for the treatment of non-typhoidal bacteraemia.[16] Clinical algorithm was developed to help in distinguishing typhoid and malaria in a hospital-based study during an epidemic of typhoid in the highlands of Papua New Guinea. In this study, mainstay of treatment was CPL and a very few problems were encountered with its use in patients.[17]

Among all currently used antimalarials, CQ is cheap, relatively well tolerated, can be administered simply and is the drug of choice in most areas, but its use as a first line treatment is now

becoming limited due to evolution of CQ resistant *P. falciparum*.[19, 20] In CQ resistant areas, its use can be extended by combining with other antimalarial drugs. The combination therapy is a present day demand to treat drug resistant malaria cases, it offers hope for preserving the efficacy of currently used antimalarials and prolonging their useful therapeutic life.[6] Quinine, a promising antimalarial drug is also being given with other antibacterial drugs having antimalarial activity, such as clindamycin, erythromycin, rifampicin and chloramphenicol.[5] Chloramphenicol, an effective antibiotic, also possessing antimalarial activity could be useful in combination with non-curative but rapidly acting antimalarial in the treatment of drug resistant malaria.

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Short Notes

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Relative Efficacy of Five Synthetic Pyrethroids against Four Vector Mosquitoes, *Anopheles culicifacies, Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti*

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Keywords: *Aedes aegypti, Anopheles culicifacies, An. stephensi, Cx. quinquefasciatus,* Efficacy, Pyrethroids

Synthetic pyrethroids are being used as alternatives to control DDT, HCH and malathion resistant malaria vectors in India,[1,2] though cross-resistance between DDT and synthetic pyrethroids in some mosquitoes due to knock-down resistance (kdr) factor, have been reported.[3–5] The property of rapid knock-down action and excito-repellency against mosquitoes coupled with low mammalian toxicity make synthetic pyrethroids as ideal molecules for vector control. Photostable analogues of synthetic pyrethroids such as deltamethrin, lambdacyhalothrin, cyfluthrin, permethrin, etc. are highly effective as residual insecticides and are used for impregnation of mosquito nets and curtains.[6–9] A contact period of few minutes with pyrethroid treated surface is sufficient to kill mosquitoes,[8] whereas in case of other insecticides the duration of contact with treated surface is much higher. Various insecticides of the synthetic pyrethroid group are now available and are being used or likely to be used in control of mosquito vectors in the coun-

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try. This paper reports the efficacy (LT_{50} values) of five synthetic pyrethroids against adult mosquitoes of laboratory colonized strains of *Anopheles culicifacies, An. stephensi, Culex quinquefasciatus* and *Aedes aegypti,* which have developed varying degree of resistance to DDT and malathion but they are still susceptible to pyrethroids and carbamates. However, there are some reports which have shown reduced susceptibility or resistance to pyrethroids in *An. culicifacies.*[10,11]

Laboratory colonized, pyrethroid susceptible strains of *An. culicifacies* sp C, *An. stephensi, Cx. quinquefasciatus* and *Ae. aegypti,* being maintained in the insectary of Malaria Research Centre for at least five years without selection pressure of any insecticide, were used to determine the baseline toxicity (in terms of lethal time) against five synthetic pyrethroids.

Insecticide impregnated papers of WHO recommended[12] diagnostic doses of deltamethrin (0.05 per cent), lambdacyhalothrin (0.05 per cent), cyfluthrin (0.15 per cent), permethrin (0.75 per cent) and bifenthrin (0.1 per cent, not a diagnostic dose), were used for bioassays which were performed with the WHO susceptibility test kit on adult mosquitoes.[13] For all these bioassays 3 to 4 days old, blood fed mosquitoes were exposed to insecticide treated papers in batches of 15 mosquitoes per tube in three replicates for each exposure time, which varied between 0.5 and 60 min and at least five different exposure times were used for each species, depending upon the mortality in each species. After the exposure for specified duration, the mosquitoes were transferred to the holding tube for recovery and the mortality was finally recorded after 24 h of the holding period. For control assay, the mosquitoes were exposed on control paper for one hour and then transferred to the holding tube for 24 h. During this period the mosquitoes in holding tubes were kept under constant temperature of $27\pm2^{\circ}C$ and relative humidity of 75±5 per cent. The data on per cent mortality vs exposure time for each mosquito species against five insecticides were analyzed using log-probit method[14] to determine their LT_{50} values. The efficacy of different pyrethroids was statistically compared in terms of LT_{50} values and their 95 per cent confidence limits. The difference was considered significant if there was no overlapping of the 95 per cent confidence limits.

Table 1 shows the relative efficacy in terms of lethal time for 50 per cent mortality (LT_{50} values) of different synthetic pyrethroids against *An. culicifacies, An. stephensi, Cx. quinquefasciatus* and *Ae. aegypti.* The LT_{50} value for *An. culicifacies,* against deltamethrin (0.05 per cent), lambdacyhalothrin (0.05 per cent), permethrin (0.75 per cent), cyfluthrin (0.15 per cent) and bifenthrin (0.1 per cent) were < 0.5, < 0.5, 0.74, < 0.5 and 1.78 min respectively, while these values for *An. stephensi* against five pyrethroids were 2.4, 7.89, 2.59, 2.955 and 29.319 min, respectively. The LT_{50} values for *Cx. quinquefasciatus* and *Ae. aegypti* against five pyrethroids were 5.169, 12.77, 11.92, 6.412 and 29.319 min and 2.364, 17.32, 2.859, 2.698 and 27.819 min, respectively.

The data revealed that of the four mosquito species, *An. culicifacies* was most susceptible to

Table 1. Relative efficacy of five pyrethroids against *An. culicifacies, An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus*

Insecticide (Conc.)	Mosquito spp	Lethal time (LT_{50}) in min (95% confidence limits)	Regression equation	χ^2 (degree of freedom)
Deltamethrin	*An. culicifacies*	<0.5	Y=2.5696X+2.79	0.2118 (2)
(0.05%)	*An. stephensi*	2.4 (1.65–3.3)	Y=0.921X+3.01	10.06 (5)
	Cx. quinquefasciatus	5.169 (3.678–6.827)	Y=1.541X+3.901	4.15 (4)
	Ae. aegypti	2.364 (1.737–3.031)	Y=1.434X+4.464	4.207 (4)
Lambdacyhalothrin	*An. culicifacies*	<0.5	Y=1.9012X+3.008	6.501 (3)
(0.05%)	*An. stephensi*	7.891 (5.888–11.859)	Y=1.278X+3.853	7.72 (3)
	Cx. quinquefasciatus	12.77 (9.398–17.029)	Y=1.557X+3.277	3.403 (3)
	Ae. aegypti	17.234 (14.014–21.508)	Y=1.7314X+2.855	4.00 (3)
Permethrin	*An. culicifacies*	0.743 (0.21–1.024)	Y=3.1542X+0.2016	0.211 (2)
(0.75%)	*An. stephensi*	2.59 (1.513–3.621)	Y=1.5695X+4.35	2.3428 (4)
	Cx. quinquefasciatus	11.92 (9.057–15.28)	Y=1.809X+3.053	0.534 (3)
	Ae. aegypti	2.859 (2.245–3.447)	Y=2.587X+3.819	8.9038 (4)
Cyfluthrin	*An. culicifacies*	<0.5*	–	
(0.15%)	*An. stephensi*	2.955 (1.385–4.584)	Y=1.064X+4.49	2.7168 (4)
	Cx. quinquefasciatus	6.412 (4.096–8.661)	Y=1.623X+3.69	0.680 (3)
	Ae. aegypti	2.698 (1.338–3.692)	Y=2.464X+3.938	0.917 (3)
Bifenthrin	*An. culicifacies*	1.7821 (0.852–2.565)	Y=2.1422X+4.462	1.195 (2)
(0.1%)	*An. stephensi*	36.251 (27.88–50.03)	Y=1.1827X+3.156	15.283 (2)
	Cx. quinquefasciatus	29.319 (22.886–39.946)	Y=1.984X+14.46	3.319 (2)
	Ae. aegypti	27.819 (21.996–36.376)	Y=1.6803X+2.573	6.1429 (2)

*100% mortality was observed in 30 sec.

all the synthetic pyrethroids tested in this study. However, the toxicity of different pyrethroids against other mosquito species varied between different species. In an earlier study adult mosquitoes of *An. culicifacies* were reported as most susceptible of the four species to K-othrine, a formulation of deltamethrin, but in the larval stage, *Culex* and *Aedes* spp were more susceptible than *An. culicifacies.*[15]

The efficacy of lambdacyhalothrin was comparable with deltamethrin against *An. culicifacies* but against other mosquito species, deltamethrin was more toxic than lamdacyhalothrin. The difference in the LT_{50} values of two insecticides was 3.28 fold higher against *An. stephensi,* 2.47 fold higher against *Cx. quinquefasciatus,* and 7.32 fold higher against *Ae. aegypti* when compared to that of *An. culicifacies.* However, there was not much difference in the LT_{50} values of deltamethrin and cyfluthrin against all the four mosquito species though cyfluthrin produced 100 per cent mortality against *An. culicifacies* in just 30 sec exposure period. Since the toxicity of

deltamethrin was determined with 0.05 per cent papers and it was more or less same as that of cyfluthrin determined with 0.15 per cent papers, it can be considered that deltamethrin is relatively more effective against different mosquito species than cyfluthrin except against *An. culicifacies*. Similarly, the LT_{50} of permethrin (0.75%) and deltamethrin (0.05%) was more or less of the same order except for *Cx. quinquefasciatus*. This clearly shows that deltamethrin is about 15–35 fold more effective than permethrin at LT_{50} level against different mosquito species. The difference in the efficacy against *Cx. quinquefasciatus* was more pronounced than other mosquito species.

Of all the five synethic pyrethroids, bifenthrin was virtually least effective, however, when the efficacy in terms of active ingradient (ai) value was compared, bifenthrin was found to be more toxic than permethrin against *An. culicifacies* and *Cx. quinquefasciatus* but less toxic than permethrin against *An. stephensi* and *Ae. aegypti*. Though the inherent toxicity of different pyrethroids differs against different species, their efficacy in the field against different mosquito species depends upon several other factors.[16,17] The data against different mosquito species, as determined in this study, clearly showed that cyfluthrin is the most effective against *An. culicifacies* followed by deltamethrin, lambdacyhalothrin, bifenthrin and permethrin, while deltamethrin is the most effective against all the mosquito species except *An. culicifacies* followed by cyfluthrin and lambdacyhalothrin, and permethrin is least effective against *An. culicifacies* and *Cx. quinquefasciatus*, while bifenthrin is least effective against *An. stephensi* and *Ae. aegypti* among different synthetic pyrethroids used in this study.

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Detection of *Plasmodium falciparum*–IgG and Incidence of Asymptomatic Malaria in Pregnant Women in Nigeria

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Keywords: Asymptomatic malaria, Gestational ages and parity, *Plasmodium falciparum*–IgG, Pregnant women

Susceptibility of pregnant women to malaria infection has been reported to change according to the age of gestation, parity[1,2] and hormonal changes encountered during pregnancy.[3] However, non-pregnant adults residing in malaria endemic areas[4–6] have shown age-related immunity against malaria. This immunity was shown to limit parasitaemia thereby making it possible for these adults to appear apparently healthy. Hence, the present study was designed to investigate the incidence of asymptomatic malaria and detect *Plasmodium falciparum* specific-IgG in pregnant women showing no sign and symptom of clinical malaria infection.

For this study 229 apparently healthy pregnant women (at different gestational age and gravidity), aged 20–35 years without any sign and symptom of malaria were recruited for the study. The pregnant women were screened for malaria parasites and separated into asymptomatic malaria (n = 139) and uninfected (n = 90) groups based on either positive or negative blood films for malaria parasites. Other tests performed with the blood samples include malaria parasites density as described by Rooth *et al.*[7] and *P. falciparum* specific (*Pf*)-IgG detection by indirect ELISA technique (Cellabs Pvt. Ltd., Australia). The mean (±SD) of the variables was determined and ANOVA was used to assess the level of significance.

The present study observed no significant difference in the malaria parasites density amongst

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the pregnant women with asymptomatic malaria in different gestational age ($p > 0.2$) or gravidity ($p > 0.2$). However, the median, mode and peak asymptomatic malaria parasites were 341, 347 and 1380/ µl of blood respectively in the asymptomatic pregnant women. The *Pf*-IgG concentration was significantly different between the asymptomatic malaria and uninfected pregnant women during the first ($p < 0.05$) and third ($p < 0.05$) trimesters of pregnancy and between the asymptomatic malaria and uninfected secondigravidae pregnant women ($p < 0.05$) (Tables 1 and 2).

The present study observed asymptomatic malaria parasitaemia amongst apparently healthy

Table 1. Log_{10} (mean ±SD) MPD and (mean ± SD) serum absorbance of *P. falciparum* specific (*Pf*)-IgG in pregnant women at different trimesters

Variables	Log_{10} mean MPD in PAM	*Pf*-IgG in PAM	*Pf*-IgG in PWM	*Pf*-IgG in PAM vs PWM
I trimester (n)	2.480±0.274 (n=46)	0.546±0.185 (n=46)	0.630±0.171 (n=30)	$p < 0.05$
II trimester (n)	2.514±0.270 (n=47)	0.603±0.289 (n=47)	0.519±0.289 (n=30)	$p > 0.2$ (NS)
III trimester (n)	2.532±0.200 (n=46) $p > 0.1$ (NS)	0.593±0.199 (n=46)	0.471±0.207 (n=30)	$p < 0.01$

SD—Standard deviation; PAM—Pregnant women with asymptomatic malaria; PWM—Pregnant women without malaria; MPD—Malaria parasite density; n—Number of subjects; NS—Nonsignificant.

Table 2. Log_{10} (mean ±SD) MPD and (mean ± SD) serum absorbance of *P. falciparum* specific (*Pf*)-IgG in pregnant women of different gravidae

Variables	Log_{10} mean MPD in PAM	*Pf*-IgG in PAM	*Pf*-IgG in PWM	*Pf*-IgG in PAM vs PWM
Primigravidae (n)	2.346±0.238 (n=48)	0.549±0.168 (n=48)	0.588±0.280 (n=30)	$p > 0.2$ (NS)
Secondigravidae (n)	2.2814±0.301 (n=37)	0.584±0.206 (n=37)	0.456±0.222 (n=30)	$p < 0.05$
Multigravidae (n)	2.316±0.200 (n=54) $p > 0.1$ (NS)	0.645±0.161 (n=54)	0.679±0.104 (n=30)	$p > 0.2$ (NS)

SD—Standard deviation; PAM—Pregnant women with asymptomatic malaria; PWM—Pregnant women without malaria; MPD—Malaria parasite density; n—Number of subjects; NS— Nonsignificant.

pregnant women. This observation suggests that pregnant women have the ability to limit malaria parasitaemia which may have been due to the gradual built-up of malaria immunity acquired over time in malaria endemic area.[7,8–11] However, the sustenance or collapse of this protective immunity may result in asymptomatic or clinical malaria respectively. Thus, this calls for the possible existence of asymptomatic malaria parasite threshold value, within which the pregnant women remain asymptomatic and outside which clinical presentation occurs. In this study the asymptomatic threshold is 1380 parasites per μl of blood, which represents +4 standard deviation of the mean parasites density. Such belief has been reported in children elsewhere.[12–14] The evidence of sustained malaria-specific antibody immunity was observed in the asymptomatic pregnant women studied. This seems stronger during the first trimester possible due to the influence of attained pre-pregnancy immunity and the decline by third trimester may be due to active inhibition of the parasite multiplicity and/or physiologic transfer to the foetus *in utero*. Therefore, the present study indicates that at certain parasites density the pregnant women may have the potential needed to limit malaria parasitaemia when compared to their counterpart nonpregnant adults.

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Situation of Malaria in Forest-fringed Villages of North Lakhimpur District, Assam

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Keywords: Assam, Forest-fringed villages, Malaria, *Pf* per cent, SPR

Malaria still remains as an important vector-borne disease in India. Despite the DDT spray operation under National Anti Malaria Programme (NAMP) as an antimalaria measure, the transmission of malaria continues to be uninterrupted[1] and causes deaths almost every year.[2]

Focal outbreaks of malaria are of common occurrence in forest-fringed villages of Assam. Morbidity and mortality due to *Plasmodium falciparum* infection is alarming.[2] Perennial and persistent transmission of malaria is well-known in this region which is compounded by *Pf* resistant to antimalarial drugs,[3,4] high man-vector contact, favourable breeding conditions, difficult terrain, lack of awareness and low socio-economic conditions. The dominance of *P. falciparum*, a killer parasite in Assam and *Anopheles minimus*, the major vector of malaria, support the continued transmission of the disease.[1,5,6] Incrimination of *An. dirus* from Dibrugarh district,[7] *An. fluviatilis* from Boko area[8] and *An. culicifacies* from Garubandha area of Sonitpur district[9] of Assam as malaria vectors, also confirm their role in the transmission of the disease. Moreover, vast ecological changes have been taken place in the region in recent years causing enormous increase in mosquitogenic conditions.

In this communication, situation of malaria and vector species in forest-fringed villages of North Lakhimpur district (Assam) are presented.

The study area is comprised of four forest-fringed villages—Durpang, Bishnupur, Santipur and Mirigaon on Assam-Arunachal Pradesh border under Dhalpur PHC in North Lakhimpur

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district of Assam. The villages are scattered, thinly populated and remote, and often located along the perennial streams and kutcha nallahs at foothills, whereas paddy fields are in flat lands. The inhabitants comprise of Assamese, ex-tea garden labourers, adivasees and tribes both from Assam and Arunachal Pradesh. The area is covered with large forestations intersected by streams and kutcha nallahs. Human dwellings are often with an adjacent cattleshed and are made of bamboos with thatched roofs. Mosquito fauna is rich and vector density is high due to frequent rainfall and conducive climate throughout the year.

Door-to-door collection of blood samples was carried out in four affected forest-fringed villages, according to active case detection (ACD). Blood samples (both thick and thin smears) were stained with Giemsa and examined under the microscope. Presumptive treatment was given to all fever cases during collection of blood samples followed by radical treatment to all malaria positive cases as per NAMP policy[10] by malaria workers to check further transmission of the disease. Epidemiological parameters such as SPR, SfR, *Pf* per cent and age-wise distribution of malaria cases were calculated.

Adult mosquitoes were collected from human dwellings and cattlesheds with the aid of 6-volt motorcycle battery-operated CDC miniature light-traps (Communicable Disease Centre, USA) from dusk- to-dawn.[6] Mosquitoes were identified using standard keys.[11] Alive mosquitoes were dissected to determine their physiological age and sporozoite infection of salivary glands.[12]

Examination of blood samples revealed 46.5 per cent positivity rate (SPR) ranging between 19.2 and 60.2. *P. falciparum* was the dominant species and accounted for 60.4 per cent of the total cases (Table 1). This confirmed the earlier observations made in different geographical areas of Assam.[1,5] Among the four villages, the highest SPR was recorded in Bishnupur (60.2) followed by Santipur (58.5), Mirigaon (38.1) and lowest in Durpang (19.2). There was little difference in the incidence of malaria among male and female populations. Maximum number of positive cases (57.5 SPR) were recorded

Table 1. Results of active surveillance of malaria in forest-fringed villages of North Lakhimpur district (Assam)

Village	Population	BSC/E	*Pf*	*Pv*	SPR	SfR	*Pf*%
Durpang	180	52	7	3	19.2	13.5	70.0
Bishnupur	260	93	83	18	60.2	40.9	67.9
Santipur	175	41	10	14	58.5	24.4	41.7
Mirigaon	145	42	9	7	38.1	21.4	56.3
Total	760	228	64	42	46.5	28.1	60.4

among population below 20 yrs of age with 63.8 per cent *Pf* cases (Table 2). Children between 6 and 15 yrs of age were the worst sufferers (63.2 SPR). Dutta and Rajvir[13] also reported high incidence of malaria among children up to 14 yrs of age in north Indian districts and Das *et al*.[6] in Nalbari district of Assam.

In an entomological survey, 11 species of anophelines and 16 species of culicines were collected from human dwellings and cattlesheds in eight trap nights. Per trap night density of mosquitoes in human dwellings and cattlesheds were 293 and 839.3 respectively (Table 3). Anopheline mosquitoes were 36.7 per cent of

Table 2. Incidence of malaria among different age groups

Age group (yrs)	BSC/E	*Pf*	*Pv*	SPR	SfR	*Pf*%
1–5	21	7	3	47.6	33.3	70.0
6–10	48	22	8	62.5	45.8	73.3
11–15	28	8	10	64.3	28.6	44.4
16–20	37	11	8	51.4	29.8	58.0
>21	94	16	13	30.9	17.0	55.2
Total	228	64	42	46.5	28.1	60.4

Table 3. Mosquitoes collected by CDC light-trap in forest-fringed villages

Mosquitoes species	No. collected				Total	
	Human dwellings		Cattlesheds			
Anopheles aconitus	20	(5)	68	(17.0)	88	(11.0)
*An. annularis**	36	(9.0)	106	(26.5)	142	(17.8)
An. barbirostris	36	(9.0)	135	(33.8)	171	(21.4)
An. crawfordi	82	(20.5)	323	(80.8)	405	(50.6)
*An. culicifacies**	18	(4.5)	54	(14.5)	72	(9.0)
An. jamesi	–		11	(2.8)	11	(1.4)
An. kochi	26	(6.5)	91	(22.8)	117	(14.6)
*An. maculatus**	52	(13.0)	175	(43.8)	227	(28.4)
*An. minimus**	29	(7.3)	115	(28.8)	144	(18.0)
*An. philippinensis**	68	(17.0)	143	(30.8)	211	(26.4)
An. vagus	26	(6.5)	46	(11.5)	72	(9.0)
Culex spp	779	(19.7)	2090	(522.5)	2869	(358.6)
Total	1172	(293)	3357	(839.3)	4529	(566.1)

*Malaria vectors encountered; Figures in parentheses indicate per trap night collection.

the total collection with 98.3 and 316.7 per trap night density in human dwellings and cattlesheds respectively. Malaria vectors encountered were *Anopheles annularis* (142), *An. culicifacies* (72), *An. maculatus* (227), *An. minimus* (144) and *An. philippinensis* (211), which formed 48 per cent of the total anophelines collected. Highest collection of malaria vectors was made from cattlesheds than human dwellings. Similar observations were also made from other parts of northeastern region.[6,14] *An. minimus,* being the major vector of malaria in northeastern region of India, played the important role in the present transmission of malaria in this area and formed 18.1 per cent of the total malaria vectors encountered.

However, the role of *An. maculatus* and *An. philippinensis,* the known vectors of malaria of this region,[15] in the transmission of the disease cannot be ruled out. *An. culicifacies,* which has recently being incriminated as a malaria vector from Garubandha area in Sonitpur district of Assam[9] has the possibility of playing a supporting role in the transmission of the disease. Dissection of malaria vectors revealed high parity rate (73.3 per cent) ranging between 70.1 and 77.6, which gives a strong indication about their vectorial status in the transmission of the disease.[13]

Presence of high percentage of parasitic load in the community and high vector density, make the population more vulnerable for contacting malaria, as locals are not in the habit of using mosquito nets or any other personal protection measures. Repeated infections and non-clearance of parasites from the blood due to underdosage of antimalarial drugs are the causes of development of immunity and asymptomatic carriers in the community. The villagers lack sense of hygiene and awareness about malaria and to achieve community compliance in public health programme, health education is urgently required in this area. Unlike other parts of the country, the major malaria vectors are still susceptible to four per cent DDT in Assam.[1,6] So, use of impregnated bednets, reasonable coverage and methodical indoor residual spray, coupled with reduction of parasitic load in the community through surveillance, timely antimalarial measures and malaria awareness camps can certainly improve the situation in this area.

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LETTER TO THE EDITOR

Indian Journal of Malariology
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Detection of Two Cases of *Plasmodium malariae* in Jharkhand

Sir — *Plasmodium malariae* causing quartan malaria is global in distribution and ranks third in incidence among other Plasmodium spp. (*P. vivax* and *P. falciparum*). *P. malariae* has been well-documented from Indonesia[1] and West Indies.[2] It has also been reported from India time-to-time. For example, three cases of *P. malariae* from Kerala[3] and one case from Assam[4] have been reported. Till date there is no documentation of *P. malariae* from Jharkhand state in India. However, in its border state — Orissa *P. malariae* infection exists 1–3 per cent of all malarial cases.[5] This state is endemic for *P. vivax* and *P. falciparum* for years. This report of *P. malariae* is the first of its kind from this region. Two cases of *P. malariae* have been detected from Daltonganj town of Jharkhand area (Fig. 1) during August to December 2002.

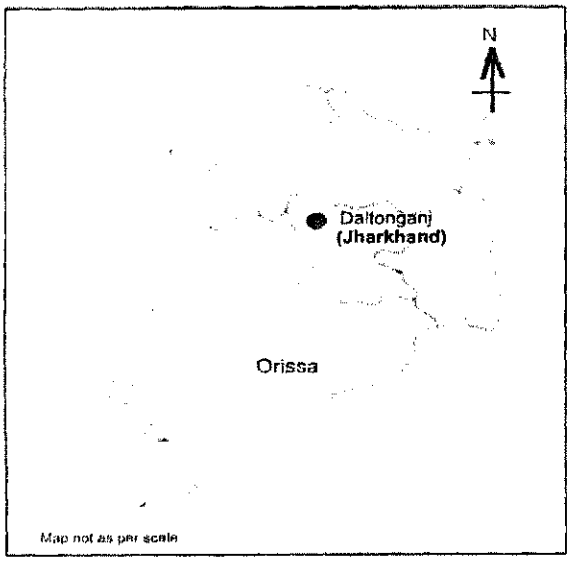

Fig. 1: Map showing location of Daltonganj (Jharkhand) and border state—Orissa

Case No. 1: A 14-year old boy complained of fever having quartan pattern for the last one and half a month. He had no history of travel outside the home town and also of blood transfusion. His liver and renal functions were normal. Spleen was not palpable. Haemoglobin was 10 g/dl. Full blood counts were normal. Peripheral blood smear (thin film) showed trophozoites of *P. malariae* (band form, ring form and mature schizont having eight merozoites) 1–5 per 100 hpf in normal sized RBCs (under oil immersion lens).

Case No. 2: A 35- year old female patient presented with fever off and on for the past one month. She was not sure of pattern of fever.

She denied ever travelling outside her home town. There was no history of blood transfusion. Liver and renal functions were normal. Haemoglobin was 8.5 g/dl. FBCs were normal. Peripheral blood smear (thin film) showed trophozoites of *P. malariae* (band form, ring form and mature schizont having eight merozoites) 4 to 10 per 100 hpf in normal sized RBCs (under oil immersion lens).

Both cases were diagnosed as *P. malariae* infections which are not known to exist in Jharkhand state till date. Though Jharkhand state, in particular Daltonganj town is known to be the endemic zone for both vivax and falciparum malaria. During August to December 2002 out of 2748 slides of fever cases, nine per cent cases were positive for trophozoite of *P. falciparum* and 9.6 per cent for *P. vivax* (P.L. Pandey—Personal communication). *P. malariae* can remain asymptomatic for years. The epidemiological significance of asymptomatic cases of *P. malariae* has been well-documented during outbreaks. Subclinical infection may be the imported source, from border state – Orissa, which has led to the development of two cases of *P. malariae* in Daltonganj. In subclinical or asymptomatic cases, levels of parasitaemia are not high enough to be detected in peripheral blood smear. Antibody detection by immunofluoroscence method screens those cases. *P. malariae* causes splenomegaly and nephrotic syndrome as long-term complications.

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A Note from the Editor: *P. malariae* infection was very common in all hyper endemic areas, viz. N.E. region, U.P. Terai, Valkanad area of Karnataka, Wynad area of Kerala and Ranchi plateau (Jharkhand). However, the infection largely disappeared during DDT era, as the extrinsic incubation period in the vector is much longer (15–20 days) than other two species and long-lived population could not survive due to DDT spray. Extensive deforestation further eroded/eliminated the ecological niches suitable for transmission of *P. malariae.* Therefore, *P. malariae* infection has become scarer in the country. However, this study presents two indigenous cases representing the few lingering ecological niches where *P. malariae* has a chance for transmission.

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Malaria Research Centre, 2-Nanak Enclave, Delhi–110 009

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