INDIAN JOURNAL OF MALARIOLOGY

Volume 37 **Numbers 1–2**

March–June 2000

MALARIA RESEARCH CENTRE
Indian Council of Medical Research
22 Sham Nath Marg
Delhi–110054

INDIAN J. MALARIOL.

Quarterly

Year of Revival: 1981

SUBSCRIPTION RATE

Annual	India	Rs. 150.00*
	Other countries (including air mail postage)	US$40.00

*25 per cent discount would be admissible to individual subscribers on annual basis.

Subscription may be made by a **demand draft** or **cheque** drawn in favour of the **"Director, Malaria Research Centre, Delhi"**, payable at Delhi and sent to the Managing Editor, *Indian Journal of Malariology*, Malaria Research Centre, 20 Madhuvan, Delhi-110 092.

The *Indian Journal of Malariology* is indexed by *BIOSIS, Drugs and Pharmaceuticals Current Indian Titles, EMBASE/Excerpta Medica, Index Medicus, Indian Science Abstracts, Review of Applied Entomology, Protozoological Abstracts, Quarterly Bibliography of Major Tropical Diseases* and it is selectively abstracted by the *Tropical Diseases Bulletin*. This journal is also accessible on the CAB Computer Database, ExtraMed CD-ROM, SourceOne UnCover and MEDLINE.

INDIAN JOURNAL OF MALARIOLOGY

Volume 37
Numbers 1–2
March–June 2000

CONTENTS

Case Report

Note: The editor assumes no responsibility for the statements and opinions expressed by the contributors. This issue is delayed due to unavoidable circumstances.

Indian Journal of Malariology
Vol. 37, March–June 2000, pp. 1–10.

Operational Feasibility and Efficacy of Deltamethrin Impregnated Hessian Curtains in Comparison to HCH Indoor Residual Spraying to Control Malaria in Selected Villages of District Ghaziabad (U.P.), India

M.A. ANSARI[a] and R.K. RAZDAN[a]

A study was carried out in selected villages of District Ghaziabad to evaluate the operational feasibility and efficacy of hessian curtains impregnated with deltamethrin @ 100 mg/m^2 in comparison to indoor residual spraying of HCH @ 0.2 g/m^2. The impregnation was carried out before onset of transmission and observations were continued up to two transmission periods. District Health Authorities have carried out HCH indoor residual spraying (IRS) as per schedule in the control village. Entomological evaluation revealed 87 per cent reduction of *An. culicifacies* up to six months in comparison to HCH indoor residual spraying. However, the reduction in densities of total mosquitoes was only 61.6 per cent. Follow-up studies revealed that the impact of deltamethrin impregnated curtains was diluted after 6–7 months. The results of bioassay tests revealed 100 per cent mortality up to 6–7 months. Epidemiological evaluation revealed 81.9 per cent reduction in total malaria cases as against 88.5 per cent with *P. falciparum* cases. Similar reduction was also observed when slide positivity rate (SPR), slide falciparum rate (SfR), cases/000 and *Pf*/000 were compared to corresponding village. Pilot studies are indicated to evaluate the relative efficacy of impregnated curtains, which is quite cheaper than conventional residual insecticide spraying (IRS).

Keywords: Deltamethrin impregnated curtains, Indoor residual spraying, Malaria control

INTRODUCTION

The strategy of malaria control under National Anti Malaria Programme (NAMP) is to carry out selective indoor residual spraying with commonly used insecticides in rural areas showing > 2 annual partasite incidence (API). However, due to development of resistance to DDT, HCH

[a]Malaria Research Centre (ICMR), 20 Madhuvan, Delhi–110 092, India.

in almost all states and malathion resistance in certain states like Maharashtra, Gujarat and Haryana synthetic pyrethroids have been introduced in recent past to tackle multi-resistant population of *An. culicifacies*, which is responsible for about 70 per cent of the malaria transmission in northern plains of rural and peri-urban areas.[1] In view of increasing cost of insecticide replacement and operational cost, it is not economically viable to promote indoor residual spraying of synthetic pyrethroids in blanket manner and allow to contaminate the environment. Personal protection measures have been successfully promoted in India and elsewhere to reduce pesticide burden and operational cost.[2,3] Though, insecticide treated mosquito nets (ITMN) have demonstrated impact on the incidence of malaria and found marginally superior than DDT indoor residual spraying in trials carried out in Solomon Islands,[4,5] however, they were not self sustainable because of only about 50 per cent reduction in malaria incidence, varying biting rhythm of vector species, non-availability of insecticide for reimpregnation, storage, sleeping habits, agriculture and forest based economy etc.[6,7] Present study was, therefore, directed to evaluate the feasibility of using deltamethrin treated hessian curtains in selected villages of District Ghaziabad for malaria control in comparison to conventional indoor residual spraying.

MATERIALS AND METHODS

Study sites

Field trials were carried out in Sidhipur and Jadoanpur villages of District Ghaziabad (Uttar Pradesh) located on the banks of upper Ganga canal about 45 km southeast of Delhi. The population of Sidhipur and Jadoanpur villages were 680 and 693 distributed in 112 and 120 houses respectively. Human dwellings are of brick walled structures consisting of one/two/three rooms (approx 3.5 x 4 m) with one main door and 1–2 windows. By and large, people sleep indoors in different seasons because of security reasons except in extreme summer (non-transmission period—May to June) when they sleep on rooftop. However, cattlesheds were mud walled and thatched roof structures. The main profession of inhabitants is agriculture. The temperature and relative humidity are variable in each season. In summer, temperature ranges from 21.5 to 42°C with 15 to 93 per cent relative humidity as against 19.6 to 40.7°C during monsoon with relative humidity of 34 to 80 per cent. Sidhipur village was selected for deltamethrin impregnated hessian curtains and Jadoanpur was taken as control, where HCH @ 0.2 g/m^2 is being sprayed by District Health Authorities in spite of precipitation of resistance.

Samples of deltamethrin (2.8 per cent) EC were obtained through the courtesy of M/S. Hoechst Agro Evo Ltd., Mumbai, India for the impregnation of curtains. Initially, health education was imparted to create awareness and motivate the community to participate in the programme. They were advised to keep their doors and windows covered with curtains for at least one hour during dusk and dawn. Prior to impregnation, measurement of curtains to be installed on windows and doors was carried out and the community was asked to cut, stitch and fix required size of curtains on the doors and win-

dows only in human dwellings. It may be pointed out that curtains were not fixed on cattlesheds, temporary structures etc. Curtains were later sprayed with the help of stirrup pump on both surfaces with deltamethrin @ 100 mg/m^2 from 27–30 June 1995. Follow-up observations were carried out up to December 1996 to evaluate whether the reduction was due to insecticide treated curtains or seasonal fluctuations.

Entomological evaluation

Indoor day time resting densities of target and non-target mosquito species were monitored before and after treatment on fortnightly basis from 0900–1300 hours in selected huts of both experimental and control villages. Collected specimens were identified in the laboratory and density per structure was calculated. The persistence of deltamethrin impregnated curtains was evaluated fortnightly by exposing laboratory reared *An. culicifacies* and *Culex quinquefasciatus*. Mosquitoes were exposed for 15 min and mortality was recorded after 24 hours as per WHO standard procedure. Corrected mortality was calculated using Abbott's formula.[8] Entries and biting rate of mosquitoes from dusk-to-dawn in human dwellings and cattlesheds were also monitored in both experimental and control villages during the post-transmission period as per procedure described earlier.[9]

Epidemiological evaluation

Epidemiological evaluation was carried out by door-to-door weekly surveillance and blood smears of all fever cases were prepared on glass micro slides and examined microscopically within 2–3 days in both experimental and control villages to assess the true incidence of malaria. Presumptive treatment was given to all fever cases, while radical treatment was given to all positive cases as per NAMP schedule.

Per cent reduction of vector densities and epidemiological indices were calculated using the following formula as described by Mulla.[10]

$$\text{Per cent reduction} = 100 - (C_1/T_1 \times T_2/C_2) \times 100$$

Where, C_1 and T_1 are pre-treatment; and C_2 and T_2 are post-treatment densities.

RESULTS

Month-wise man hour densities of *An. culicifacies* and other mosquitoes are depicted in Table 1 along with per cent reduction based on pre- and post-treatment densities of HCH sprayed village. It is clear from the table that pre-treatment density of *An. culicifacies* was more or less same in both the villages. However, impregnation of deltamethrin @ 100 mg/m^2 in Sidhipur village has resulted drastic reduction in the indoor adult density of vector species in successive months. The per cent reduction against *An. culicifacies*, *Cx. quinquefasciatus* and total mosquitoes was 87.0, 65.7 and 61.6 per cent respectively in the experimental village. The reduction in adult density was also indicated in January and February 1996 followed by steep recovery of population from March to December 1996. Similar reduction was also observed when entry and biting rate of mosquitoes was compared between the two villages. Inhabitants slept in houses treated with deltamethrin were completely protected from

Table 1. Man hour density of mosquitoes in village Sidhipur and Jadoanpur, Dhaulana PHC, District Ghaziabad (U.P.)

Month/ Year	*An. culicifacies*				Total anophelines				*Cx. quinquefasciatus*				Total mosquitoes			
	E		C		E		C		E		C		E		C	
	HD	CS	HD	CS	HD	CS	HD	CS	HD	CS	HD	CS	HD	CS	HD	CS
1995																
May	22	41	19	34	44	102	40	83	76	96	64	112	120	198	104	195
Jun	17	34	15	30	25	81	22	63	27	64	22	50	52	145	44	113
Av.	19.5	37.5	17	32	34.5	91.5	31	73	51.5	80	43	81	86	171.5	74	154
Jul	9	115	39	68	32	526	72	282	12	122	32	80	44	648	104	362
Aug	6	142	56	96	40	880	144	563	20	180	44	134	60	1060	188	697
Sep	8	173	65	132	44	1132	186	706	22	274	60	126	66	1406	246	832
Oct	9	202	82	152	96	912	172	616	27	302	101	180	123	1214	273	796
Nov	4	96	32	79	63	201	140	364	17	186	44	110	80	387	184	474
Dec	3	42	28	39	28	96	42	102	11	77	37	80	39	173	79	182
Total	39	770	302	566	303	3747	756	2633	109	1141	318	710	412	4888	1074	3343
(Av.)	(6.5)	(128.3)	(50.3)	(94.3)	(50.5)	(624.5)	(126)	(438.8)	(18.1)	(190.1)	(53)	(118.3)	(68.6)	(814.6)	(179)	(557.1)
% reduction	87.0	–36.0			59.9	–42.3			65.7	–60.7			61.6	–46.2		

contd...

Table 1. (*contd.*)

Month/ Year	*An. culicifacies*				Total anophelines				*Cx. quinquefasciatus*				Total mosquitoes			
	E		C		E		C		E		C		E		C	
	HD	CS	HD	CS	HD	CS	HD	CS	HD	CS	HD	CS	HD	CS	HD	CS
1996																
Jan	5	45	7	41	12	52	16	40	4	30	8	37	16	82	24	77
Feb	12	54	26	50	63	143	72	32	27	80	25	60	90	305	97	192
Mar	26	45	23	44	52	166	40	144	64	142	59	120	116	308	99	264
Apr	35	104	31	106	74	182	70	170	120	248	116	230	194	430	186	400
May	14	44	12	39	37	90	34	81	80	122	64	112	117	212	98	193
Jun	8	23	7	21	22	64	17	52	16	37	18	40	38	101	35	92
Jul	22	82	19	73	122	321	89	302	42	112	49	90	164	434	138	392
Aug	27	118	24	122	160	420	143	428	53	132	56	112	213	552	199	540
Sep	24	127	22	132	208	702	182	773	62	156	60	124	270	858	242	897
Oct	17	86	15	74	242	763	112	808	88	141	88	139	330	904	200	947
Nov	11	42	8	44	76	93	62	96	64	112	61	98	140	205	123	194
Dec	9	81	7	42	22	52	19	60	28	92	30	80	50	144	49	140
Total	210	851	201	788	1090	3048	856	2986	648	1404	634	1242	1738	4535	1490	4328
(Av.)	(17.5)	(70.9)	(16.7)	(65.6)	(90.8)	(254)	(71.3)	(248.8)	(54)	(117)	(52.8)	(103.5)	(144.8)	(377.9)	(124.1)	(360.6)
% reduction	–4.4	–7.9			–27.3	–2.0			–2.2	–13.0			–16.6	–4.7		

E — Experimental, where deltamethrin impregnated curtain @ 100 mg/m^2 were used; C — Control first round of HCH was sprayed @ 200 mg/m^2; HD — Human dwellings; CS — Cattlesheds.

Table 2. Landing rate and per cent protection obtained by using deltamethrin impregnated curtain of mosquitoes in Sidhipur (E) and Jadoanpur (C) villages of Dhaulana PHC, Distt. Ghaziabad (U.P.)

Year	Experimental/ Control (E/C) villages	Species of mosquitoes					
		An. culicifacies		*Cx. quinquefasciatus*		Total mosquitoes	
		Landing rate	% protection	Landing rate	% protection	Landing rate	% protection
Human dwellings							
1995	E	0.0	100.0	4.0	89.3	3.25	88.9
	C	2.0		25.7		29.5	
1996	E	1.0	0.0	18.7	–1.3	21.2	1.1
	C	1.0		18.5		21.5	
Cattlesheds							
1995	E	10.5	–13.5	107.7	–3.1	197.7	–4.0
	C	9.2		104.5		190.0	
1996	E	8.7	14.6	90.5	15.3	166.7	–1.5
	C	10.7		78.0		165.5	

An. culicifacies bites. However, the protection against *Culex quinquefasciatus* and total mosquitoes was only 89.3 and 88.9 per cent respectively (Table 2).

Bioassay test

From the results depicted in Fig. 1, it was revealed that deltamethrin @100 mg/m^2 persisted more than six months. The per cent corrected mortality was 100 per cent after initial impregnation and remained more or less same up to eight months suggesting thereby that only one impregnation before onset of transmission is more than adequate to curtail the transmission in seasonal transmission areas.

Epidemiological impact

Month-wise epidemiological indices of experimental and control villages are given in Table 3. The results revealed that blood examination rate (BER) and cases/000 were almost comparable. However, the difference can be seen after impregnation of curtains as almost all indicators particularly *Pf*/000 in Sidhipur village. The overall reduction during transmission period of 1995 was 60.8 (SPR), 75.2 (SfR), 78.8 (cases/000) and 86.3 (*Pf*/000). However, complete transmission was not interrupted because of movement of the population and sleeping habits due to extreme hot and sultry weather during monsoon period. The results of follow-up studies during 1996 revealed that the impact of curtains was diluted and the transmission in Sidhipur village returned to the same degree as of Jadoanpur village. Nevertheless, the study revealed high degree of curtailment of transmission in the village where deltamethrin impregnated curtains were used. This substantiates

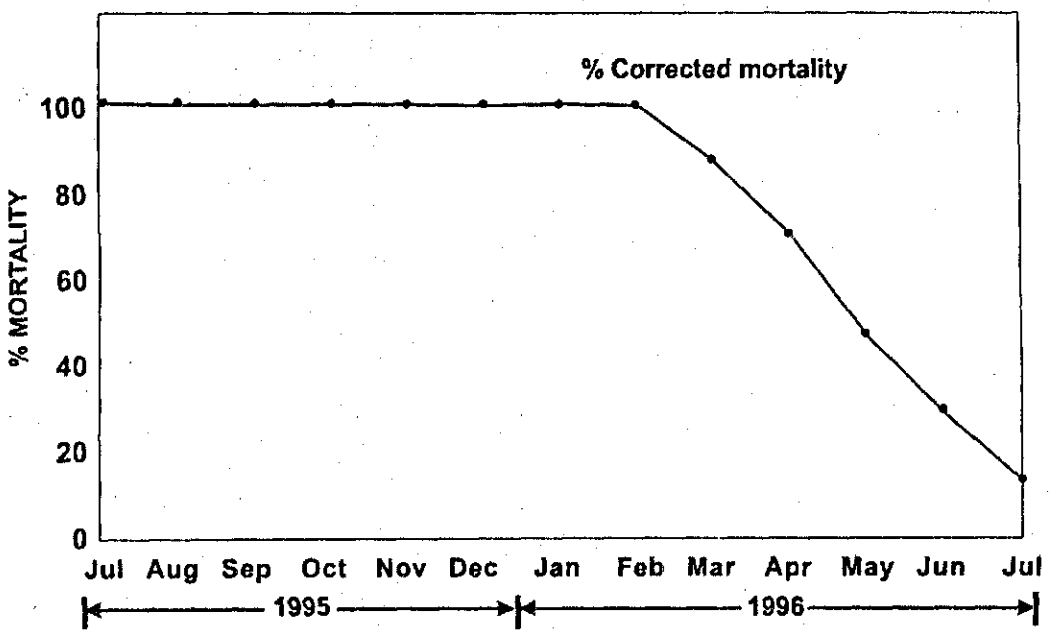

Fig. 1: Results of bioassay carried out against *An. culicifacies* in Sidhipur village

earlier findings,[11-14] who have demonstrated that impregnated curtains can substantially control vector density and the incidence of malaria in urban areas.

DISCUSSION

Malaria is a serious public health problem in India and about 2–3 million incidences are recorded annually. NAMP strategy is to carry out indoor residual spraying in rural areas showing more than two API. However due to increasing cost of insecticide and operational cost, the coverage of target population has not been achieved in recent past. This has resulted in fulminating outbreak of malaria in different parts of the country and malaria incidence particularly falciparum malaria have shown alarming increase for past several years. In view of this, one has to critically analyze the merits of indoor residual spraying in context of degraded aquatic and terrestrial environment. Probable option could be to reduce the insecticide burden on the environment by promoting personal protection measures, which are quite effective, community base and does require very small quantity of insecticide for impregnation. It may be appropriate to point out that emphasis has been placed to promote the insecticide impregnated bednets in Global Roll Back Malaria Programme. The total hessian cloth used during the present study was about 537 m averaging about 4.7 m per human dwelling costing about Rs.5000.00. Similarly, deltamethrin consumed for impregnation of 537 m hessian cloth was 1.87 litre costing about Rs.1496.00. The total

Table 3. Epidemiological indices of Sidhipur and Jadoanpur villages of Dhaulana PHC, District Ghaziabad (U.P.)

Month/ Year	SPR		SfR		Cases/000		*Pf*/000	
	E	C	E	C	E	C	E	C
1995								
May	16.6	11.1	0.0	0.0	1.47	1.25	0.0	0.0
Jun	28.5	8.3	14.2	0.0	2.9	1.25	0.0	0.0
Total (Av.)	22.5	9.7	14.2	0.0	2.1	1.25	0.0	0.0
Jul	14.8	28.5	0.0	0.0	5.88	10.0	0.0	0.0
Aug	0.0	19.4	0.0	16.4	0.0	16.3	0.0	13.8
Sep	10.7	23.3	7.1	20.9	8.8	36.4	5.8	32.6
Oct	12.1	38.1	9.0	30.9	5.8	26.3	4.4	21.3
Nov	10.2	21.2	5.1	20.3	5.8	30.1	2.9	28.8
Dec	5.8	14.4	5.8	14.4	1.4	12.5	1.4	12.5
Total (Av.)	9.0	23.0	4.7	19.0	27.9	131.9	14.7	109.2
% reduction	60.8		75.2		78.8		86.3	
1996								
Jan	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Feb	3.1	1.7	0.0	0.0	1.4	1.2	0.0	0.0
Mar	0.0	2.3	0.0	0.0	0.0	1.4	0.0	0.0
Apr	11.1	12.5	0.0	0.0	2.9	3.7	0.0	0.0
May	0.0	6.6	0.0	0.0	0.0	1.2	0.0	0.0
Jun	8.3	9.0	0.0	0.0	1.4	1.4	0.0	0.0
Jul	0.0	22.2	0.0	0.0	0.0	2.8	0.0	0.0
Aug	5.3	16.6	1.7	8.3	4.4	7.5	1.4	3.7
Sep	33.3	12.0	25.0	8.8	17.6	13.8	13.2	10.0
Oct	18.5	16.6	11.1	12.8	7.3	18.7	4.4	14.4
Nov	20.0	5.2	20.0	2.6	2.9	2.5	2.9	1.2
Dec	10.0	18.1	0.0	0.0	1.4	5.0	0.0	0.0
Total (Av.)	10.5	9.5	5.8	4.6	39.7	56.5	22.0	27.0
% reduction	–10.5		–26.0		29.7		20.2	

E — Experimental village, where deltamethrin impregnated curtains were used; C — Control village where first round of HCH was sprayed @ 200 mg/m^2 ; First impregnation with deltamethrin @ 100 mg/m^2 was carried out during 27–30 June 1995.

cost of curtain and impregnation was about Rs. 6496.00 as against Rs.11,420.00 towards the cost of HCH insecticide in Jadoanpur village. It may be pointed out that this does not include the cost of equipment and spraying of insecticide for three rounds. Besides enormous cost difference, the HCH residual spraying did not provide either reduction in adult densities of *An. culicifacies* or in the incidence of malaria. Contrary to this, deltamethrin impregnated hessian curtains has substantially reduced the adult density, man-mosquito contact and the incidence of malaria. These findings are in agreement with earlier findings.[11,14] It may also be appropriate to point out that hessian jute curtains are socially accepted and toxicologically safer because of less contact of insecticide on human body.[12] In addition to this, they also provide collateral benefits as earlier demonstrated.[11] The study revealed that there is urgent need to revise the strategy of malaria control in the context of these findings. Nevertheless, the pilot project may be initiated to evaluate the relative efficacy and cost-effectiveness of impregnated bednet, hessian cloth curtain and indoor residual spraying.

ACKNOWLEDGEMENTS

Technical assistance provided by Mr. S.N.S. Kacchawaha, Mr. Karam Chand, Mr. Janak Singh, Mr. Mahesh Sharma, Mr. M.D. Tewari, Mr. B.D. Sati, Mr. M.R. Sethi, Mrs. Santosh Vashistha, Mr. Ram Raj Singh, Mr. D.S. Jha and Mr. I.D. Roy is gratefully acknowledged.

REFERENCES

1. Ansari, M.A., V.P. Sharma, R.K., Razdan and P.K. Mittal (1990). Field evaluation of deltamethrin against resistant *An. culicifacies* in District Ghaziabad (U.P.), India. *Indian J. Malariol.*, **27:** 1–13.
2. Jana-Kara, B.R., W.A. Jiullah Khan, B. Shahi, Vas Dev, C.F. Curtis and V.P. Sharma (1995). Deltamethrin imprgenated bednets against *Anopheles minimus* transmitted malaria in Assam, India. *J. Trop Med. Hyg.*, **98:** 73–83.
3. Rozendaal, J.A. and C.F. Curtis (1989). Recent research on impregnated mosquito nets. *J. Amer. Mosq. Contr. Assoc.*, **5:** 500–507.
4. Hii, J.K.L., L. Kanoi, A. Folegela, S.K.P. Kon, T.R. Burkot and R.A. Wirtz (1993). Impact of permethrin impregnated mosquito net compared with DDT house spraying against malaria transmission by *Anopheles farauti* and *An. punctulatus* in the Solomon Island. *Med. Vet. Entomol.*, **7:** 333–338.
5. Kere, N.K., A. Arabola, B. Bakote'e, O. Qalo, T.R. Barkot, R.H. Webber and B.A. Southgate (1996). Permethrin impregnated bednets are more effective than DDT house spraying to control malaria in Solomon Island. *Med. Vet. Entomol.*, **10:** 145–148.
6. Bermejo, A. and H. Veeken (1992). Insecticide impregnated bednets for malaria control. A review of field trials. *Bull. WHO*, **70**(3): 293–296.
7. Singh, N., A.L. Mishra, O.P. Singh, A. Jaiswal and M.T. Khan (1994). Feasibility study on the introduction of insecticide impregnated bednets for malaria vector control in forest villages of District Mandla (Madhya Pradesh). *Indian J. Malariol.*, **31:** 136–140.
8. Abbott, W.S. (1995). A method for computing the effectiveness of an insecticide. *J. Econ. Entomol.*, **18:** 265–267.
9. Ansari, M.A., V.P. Sharma and R.K. Razdan (1993). Esbiothrin impregnated ropes as mosquito repellents. *Indian J. Malariol.*, **29:** 203–210.
10. Mulla, M.S. (1971). Control of chironomid midges in recreational lake. *J. Econ. Entomol.*, **64:** 300–307.
11. Ansari, M.A. (1997). Efficacy of deltamethrin impregnated hessian cloth curtains to control ma-

laria in urban slum settlement, Delhi (National Capital Territory). *Trop. Biomed.*, **14:** 27–33.

12. Majori, G., G. Sabatinelli and M. Coluzzi (1987). Efficacy of permethrin impregnated curtains for malaria vector control. *Med. Vet. Entomol.*, **1:** 185–192.
13. Mutinga, M.J., D.M. Renapurkar, D.N. Wachira, C.M. Mutero and M. Basimike (1992). Evaluation of the residual efficacy of permethrin impregnat-ed screens used against mosquitoes in Marigat, Baringo District, Kenya. *Trop. Med. Parasitol.*, **43:** 277–281.
14. Poopathe, S. and D. Raghunatha Rao (1995). Pyrethroid impregnated hessian curtains for protection against mosquitoes indoors in south India. *Med. Vet. Entomol.*, **9:** 169–175.

Indian Journal of Malariology
Vol. 37, March–June 2000, pp. 11–17.

Malaria during Pregnancy and its Effects on Foetus in a Tribal Area of Koraput District, Orissa

L.K. DAS[a]

Malaria during pregnancy and its maternal and foetal complications was studied in Koraput district of Orissa — a tribal area, endemic for malaria. A total of 209 pregnant women with 738 pregnancy months were studied. The parasitic index among the pregnant women ranged between 10.8 and 25.6 per cent with peak incidence during post-monsoon months. There was a significant difference in parasite incidence between the primi- and multigravidae ($p<0.05$) but difference was not observed between the trimesters. The mean haemoglobin (Hb) concentration declined to 8.4 g/dl (range 7.2–10.2 g/dl) at full-term and parturition from its initial level of 9.6 g /dl (range 7.2–12.8 g/dl). There was a significant difference ($p<0.05$) in Hb concentration among the trimesters of pregnancy. There was no significant difference in the outcome of pregnancies in women with or without malaria prarasites in their peripheral blood. There was no significant difference in Hb concentrations between malaria parasite positive and negative pregnant women ($p>0.05$). Significant difference was observed in the proportion of newborn positives from mothers with or without malaria parasites indicating a high degree of transplacental transmission. The overall foetal mortality rate was 21.5 per cent. The miscarriage, stillbirth, premature delivery leading to foetal and neonatal along with perinatal mortality constituted for 24.4, 13.3, 20 and 17.7 per cent of all mortalities respectively.

Keywords: Foetus, Malaria, Malaria during pregnancy

INTRODUCTION

Women are not intrinsically more susceptible to malaria and in fact antibodies against malaria are stronger in women than in men.[1] However, during pregnancy, there is apparent lack of immunity against malaria[2,3] and morbidity and mortality increase as a sequelae to malaria infection.[4-6] Malaria is a known complication of pregnancy in hyper- and holoendemic malarious areas leading to placental malfunction and explains high foetal mortality[7] and many such

[a]Vector Control Research Centre (ICMR), Medical Complex, Indira Nagar, Pondicherry–605 006, India.

pregnancies go into premature labour and still births.[5,8] Therefore, there is a need to study the problem magnitude burden of malaria in the vulnerable group of population like children and pregnant women in the areas endemic for malaria to formulate any form of control strategy. The present communication deals with the findings of the incidence of malaria in women during pregnancy and its possible maternal and foetal complications in a malaria endemic area in Koraput district of Orissa.

MATERIALS AND METHODS

Study area

The study villages are situated on the hilltop plateaus of Borigumma and Dasamantpur community health centre area of Koraput district in Orissa. The hilltop villages are hyperendemic for falciparum malaria with perennial transmission. The details of geographical situation and malaria problem have already been described.[9]

Study subjects

Women with a pregnancy of 12 weeks identified from 32 hilltop villages were included in the present study. Pregnant women with chronic diseases like diabetes, hypertension, tuberculosis, sexually transmitted diseases (STD), mental disorders or declared unfit by the physicians for any other cause were not included in the study. Diagnosis of pregnancy was done on the basis of the history of last missed menstrual period. The gravidity and parity status were determined by interrogating the women regarding their past obstetrical history and number of living children. The expected date of delivery was determined following simple obstetrical calculation. All subjects were explained about the study and their verbal consents were obtained.

Study design

Since the villages are situated on the hilltops and have poor communication facilities, follow-up of the study subjects was carried out at an interval of two months from a base camp till the women delivered their babies. Since the women were included in four different months, a cohort of pregnant women included in the first batch were followed to know the changes in malaria parasite rates and haemoglobin (Hb) concentrations in different trimesters of pregnancy. All malaria positive cases detected during the surveys were treated with conventional dosage schedule of chloroquine (25 mg/kg body weight). However, primaquine was not given due to obvious reasons.

During each survey, peripheral blood smear was collected from each subject and examined for malaria parasites as per standard procedures.[10] Blood Hb concentration was measured by colorimetric procedure by Sahli's haemoglobinometer. Progress in pregnancy, abortion, perinatal and neonatal mortality, if any was ascertained through verbal communication from women/relatives. Outcome of the pregnancy was compared between women with and without malaria parasites in their peripheral blood. The causes of foetal deaths were ascertained on the basis of clinical judgement and history elicited from mother/relatives. Peripheral blood smears from the surviving infants were taken and examined for malaria parasites.

Data analysis was carried out to know the malaria positivity (proportion of women positive for malaria parasite) in pregnant women according to their parity and trimester status. The difference was tested for significance by using chi-square test. The effect on pregnancy and its outcome was assessed in mothers positive and negative for malaria parasites.

RESULTS

A total of 209 pregnant women from 32 villages were examined during the study. The mean age of the women was 25 and varied between 18 and 35 years. The proportion of different parity status of study women is shown in Table 1. Only 20.1 per cent are primigravidae and highest (37.8 per cent) were with their second pregnancies. About 3.3 per cent of cases were grand multiparae with their sixth or more pregnancies. One case was with her eighth pregnancy.

The 209 study subjects exposed to 738 pregnancy months and the incidence of malaria was 11.6 per cent and varied between 0 and 17.5 per cent in different parities (Table 1). The parasite rates between primi- and multigravidae varied significantly ($p < 0.05$).

Table 1. Parity wise incidence of malaria in pregnant women

Parity status	Pregnant women		Total pregnancy (months at risk)	SPR
	No.	%		
P0	42	20.1	137	17.5
P1	79	37.8	233	11.1
P2	31	14.8	147	10.9
P3	32	15.3	110	11.8
P4	17	8.1	75	6.7
P5	5	2.4	27	7.4
P6	2	0.9	8	0
P7	1	0.5	1	0
Total	209	100	738	11.6

p-value for primi vs multiparae: $p < 0.05$; SPR — Slide positivity rate.

The parasite rates varied between 10.8 and 25.6 per cent in different months, the lowest being 10.8 per cent in the month of February and the highest (25.6 per cent) during post rainy months (August). The differences in the parasite rates in different months were statistically significant ($p < 0.05$) suggesting the seasonal influence of transmission (Table 2).

Trimester-wise parasite rates analyzed from the

Table 2. Month-wise incidence of malaria in pregnant women

Month	No. sampled	% (+) ve	No. multigravidae	% (+) ve	No. primigravidae	% (+) ve	p-value
June	95	11.6	71	9.8	24	16.6	0.49
Aug	78	25.6	63	23.8	15	33.3	0.55
Oct	143	21.0	124	21.8	19	15.8	0.67
Dec	117	17.1	106	16.9	11	18.2	0.81
Feb	102	10.8	92	9.7	10	20.0	0.55

cohort sample showed that the parasite rates during second trimester, full-term and parturition did not differ significantly ($p > 0.05$, Table 3).

Blood haemoglobin concentration: There was no significant difference in the Hb concentration between malaria parasite positive and negative mothers ($p > 0.05$). In the cohort, the Hb concentration decreased from initial level of 9.6 to 8.6 g/dl at parturition (Table 4).One case succumbed to severe anaemia (Hb < 7 g/dl)[11] and hypoproteinaemia during second trimester of pregnancy.

Table 3. Follow-up of a cohort of pregnant women for malaria incidence

Trimester of pregnancy	No. sampled	% (+) ve	*Pf* % (+) ve	*Pv* % (+) ve
First	95	11.6	90.9	9.1
Second	94	20.2	90.0	10.0
Third	69	13.0	66.7	33.3
PP	71	14.1	70.0	30.0

PP — Puerperium; $p > 0.05$.

Table 4. Follow-up of a cohort of pregnant women for haemoglobin concentration

Trimester of pregnancy	No. sampled	Min	Max	Mean Hb/g/dl	±SD
First	49	7.2	12.8	9.6	1.2
Second	72	7.4	12.2	9.0	1.09
Third	60	6.4	12.2	9.0	1.19
PP	42	7.6	9.6	8.6	0.46

PP — Puerperium; $p < 0.05$.

Effect of malaria on the outcome of pregnancies: Out of total 209 pregnancies, only 163 delivered full-term healthy babies out of which 30 (18.4 per cent) were infected within a month and predominant parasite was *P. falciparum* (Table 5). The probable effects of malaria on the outcome of pregnancies and malaria infection in the newborns are given in Table 6. There was no significant difference ($p > 0.05$) in the foetal mortality from mother with and without malaria parasite infection. However, one time negative blood smear and the shortcomings in the conventional microscopy for confirming the presence of malaria parasite in hyper- and holoendemic areas should

Table 5. Malaria in normal full-term newborns (Taken between 21-30 days of birth)

Newborns	163
Malaria parasite (+) ve	30
Pf (+) ve	18
Pv (+) ve	9
Pf + *Pv* (+) ve	3

Table 6. Comparison of effects of malaria on newborns

Effect on newborns	(+) ve mothers	%	(–) ve mothers	%	p-value
Foetal deaths	19	27.9	26	18.4	0.84
(+) ve	17	25.0	13	9.2	0.5
(–) ve	32	47.1	102	72.3	0.007
Total	68	100	141	100	

Table 7. Causes of foetal and newborn mortalities (n = 45)

Causes	(+) ve mothers	(–) ve mothers
Neonatal deaths	6	3
Perinatal deaths	5	3
Stillbirths	3	3
Fever	2	2
Premature delivery	1	5
Abortion	1	10
Unknown	1	0
Total	19	26

be taken into consideration. On the contrary, there was a significant difference ($p < 0.05$) between the proportion of newborn positives and negatives born from the mothers with or without malaria parasites (Table 7).

Foetal mortality: Table 7 shows the causes of foetal and newborn mortalities in malaria positive and negative mothers. Out of overall 45 deaths, 11 were due to abortion, six had still births (deaths due to prematurity), low birth weight comprised of six cases, neonatal deaths nine, perinatal deaths eight, due to fever (newborns) four and one unknown.

DISCUSSION

It has been observed that wherever malaria is unstable, pregnant women are more susceptible to severe forms of *P. falciparum* infection with exacerbation of parasitaemia.[6,12-14] Falciparum malaria during pregnancy is a dangerous disease and poses considerable management problem. The risk of maternal mortality in these patients is 2–10 fold higher than that of in non-pregnant women in unstable malaria transmission areas[13] because the abortion, still births, premature delivery and low birth weight are common. In areas with stable malaria, maternal morbidity is mainly due to anaemia and is usually due to postpartum haemorrhage[4,15] and the major effect on foetus is low birth weight.[16,17] The risk of all these complications is higher in primigravidae.

The present study area is hyper-endemic for falciparum malaria with perennial transmission and infection of malaria can not be ruled out even in the absence of malaria parasites in the peripheral blood by the conventional blood examination by light microscopy.[18] In falciparum malaria red cells containing mature forms of asexual parasites are concentrated in placenta[14,19] and hamper placental function leading to high foetal mortality[20] and premature labour.[21] In a study carried out in a tribal area in India, malaria was an important cause of more foetal deaths in pregnant women.[22] In the present study, both foetal and neonatal deaths are equally important sequelae of malaria. Absence of significant difference in the proportion of foetal and newborn mortalities from mothers with and without malaria parasites in their peripheral blood might probably because of shortcomings in the microscopic diagnosis of malaria or in the frequency of follow-up. The premature babies might have succumbed to failure in taking food and most probably due to a variety of infections during perinatal period as suggested by the presence of fever.

The parasite rate of the healthy newborns could have been more but due to maternal immunity (even though reduced) in this high endemic area might have a role in reducing the infection. However, there are higher chances for getting malaria infection in babies born to positive mothers in comparison to negative mothers but, if the role of maternal immunity is also taken into consideration, then it can be inferred that there is high degree of transplacental transmission in this tribal area.

Malaria is a known complication during pregnancy in hyper- and holoendemic malarious areas. All women of reproductive age in this area have usually acquired high degree of functional immunity against malaria. However, there is an exacerbation of falciparum malaria during pregnancy among primigravidae, which may be related to local immuno-suppression in the placenta, rather than a general breakdown of systemic immune defence.

It can be concluded that the effect of malaria in intrauterine life of foetus was evident. Lack of significant mortality in the study population indicated that they have already built up certain degree of immunity against malaria over the years in this area.

ACKNOWLEDGEMENTS

The author thanks Dr. P. Jambulingam of VCRC for correcting the manuscript and Dr. P.K. Das, Director, VCRC for allowing to undertake the study. The author acknowledges the help rendered by the technical staff of VCRC field station, Jeypore for the study.

REFERENCES

1. Molineaux, L. and G. Gramiccia (1980). *The Garki Project: Research on Epidemiology and Control of Malaria in Sudan Savanna of West Africa* (WHO, Geneva): 251.
2. McGregor, I.A., D.S. Rowe, M.E. Wilson and W.Z. Billewicz (1970). Plasma immunoglobulin concentration in African Gambian community in relation to season, malaria and other infection and pregnancy. *Clin. Exp. Immunol.*, **7:** 51–74.
3. Logie, D.E., I.A. McGregor, D.S. Rowe and W.Z. Billewicz (1973). Plasma immunoglobulin concentration in mothers and newborn children with special reference to placental malaria. Studies in the Gambia and Nigeria. *Bull. WHO*, **49:** 547–554.
4. Anon, P.J. (1979). Maternal deaths in the Kilimanjari region of Tanzania. *Trans. R. Soc. Trop. Med. Hyg.*, **73:** 284–288.
5. Greewood, B.M., A.M. Greenwood, R.W. Snow, P. Byass, S. Bennet and N'Jie A.B. Hatib (1989). The effect of malaria chemoprophylaxis given by traditional birth attendants on the course and outcome of pregnancy. *Trans. R. Soc. Trop. Med. Hyg.*, **83:** 589–594.
6. McGregor, I.A. (1984). Epidemiology, malaria and pregnancy. *American J. Trop. Med. Hyg.*, **33:** 517–525.
7. Logie, D.E. and I.A. McGregor (1970). Acute malaria in newborn infants. *British Med. J.*, **3:** 404–405.
8. Brabin, B.J, M. Ginny, M. Alpers, I. Brabin, T. Eggelte and H.J. Van Der Kaay (1990). Failure of chloroquine prophylaxis for falciparum malaria in pregnant women in Madang, Papua New Guinea. *Ann. Trop. Med. Parasitol.*, **84:** 1–9.
9. Rajagopalan, P.K, P.K. Das, S.P. Pani, P. Jambulingam, S.S.S. Mohapatra, K. Gunasekaran and L.K. Das (1990) Parasitological aspects of malaria persistence in Koraput district. *Indian J. Med. Res.*, **91**(A): 44–51.

10. Bruce-Chwatt, L.J. (1985). *Essential Malariology*, II edn. (William Heinemann Medical Books Ltd., London): 114.

11. WHO (1989). *Report of the Africa Regional Consultation on Control of Anaemia in Pregnancy* (Document AFR/MCH/86, WHO, Geneva).

12. McGregor, I.A. and D.A. Smith (1952). A health, nutrition and parasitological survey in a rural village Kenevia in West Kiang, The Gambia. *Trans. R. Soc. Trop. Med. Hyg.*, **46:** 403–427.

13. Brabin, B.J. (1983). An analysis of malaria in pregnancy in Africa. *Bull. WHO*, **61:** 1005–1016.

14. Bray, R.S. and R.E. Sinden (1979). The sequestration of *Plasmodium falciparum* infected erythrocytes in the placenta. *Trans. R. Soc. Trop. Med. Hyg.*, **73:** 716–719.

15. Greenwood, A.M., B.M. Greenwood, A.K. Bradley, K. Wiiliams, F.C. Shenton, S. Tulloch, P. Byass and F.S.J. Oldfield (1987). A prospective study on the outcome of pregnancy in a rural area of Gambia. *Bull. WHO*, **65:** 635–643.

16. Gilles, H.M., B.B. Lawson, M. Sibelas, A. Voller and N. Allan (1969). Malaria, anaemia and pregnancy. *Ann. Trop. Med. Parasitol.*, **63:** 245–263.

17. McGregor, I.A., M.E. Wilson and W.Z. Billewicz (1983). Malaria infection of the placenta in the Gambia, West Africa, its incidence and relationship to still birth, birth weight and placental weight. *Trans. R. Soc. Trop. Med. Hyg.*, **77:** 232–244.

18. Payne, D. (1988). Use and limitations of light microscopy for diagnosis of malaria at the primary health care level. *Bull. WHO*, **66:** 621–626.

19. Fried, M. and P.E. Duffy (1996). Adherence of *Plasmodium falciparum* to chondroitin sulfate A in the human placenta. *Science*, **272:** 1502–1504.

20. Covell, G. (1950). Congenital malaria. *Trop. Dis. Bull.*, **47:** 1147–1167.

21. White, N.J. and D.A. Warrell (1988). The management of severe malaria. *Malaria*, v.I, edited by W.H. Wernsdorfer and Sir I. McGregor (Churchill Livingstone, London) : 883.

22. Singh, Neeru, M.M. Shukla, R. Srivastava and V.P. Sharma (1995). Prevalence of malaria among pregnant and nonpregnant women of District Jabalpur, Madhya Pradesh. *Indian J. Malariol.*, **32:** 6–13.

Indian Journal of Malariology
Vol. 37, March–June 2000, pp. 18–26.

Effect of Plant Spacing on the Population of Mosquito Immatures in Rice Fields in Madurai, South India

T. JOHN VICTOR[a] and R. REUBEN[b]

A study was conducted during 'Kuruvai' crop season from December 1992 to January 1993 in the rice fields of the Agricultural College and Research Institute, Madurai, Tamil Nadu to determine the influence of plant spacing and plant canopy on the populations of mosquito immatures. Three paddy varieties (ADT36, IR50 and IR20) were selected with two types of plant spacing, one with normal spacing (15 x 10 cm) and another wider (20 x 15 cm) than the normal spacing. Results showed that the field planted with normal spacing of paddy had significantly higher populations of culicine and anopheline immatures than the fields planted with wider spacing of paddy. The paddy varieties did not have any significant effect on the population of mosquito immatures. Light intensity, measured at the water surface using an illuminometer, was inversely related to the development of plant canopy and the results suggested that plant canopy does not inhibit oviposition by mosquitoes in the early stages of paddy growth, but it was responsible for the decline in the populations in the later stages of paddy growth. The plant spacing had a significant effect on the populations of chironomids and libellulids and other insects were not affected significantly.

Keywords: Mosquito immatures, Paddy varieties, Plant canopy, Plant spacing, Rice fields

INTRODUCTION

Mosquito larval densities have been found to differ drammatically from one field to another, with only a few rice fields being major breeding sites.[1] The density of mosquito immatures in the rice fields is mainly influenced by oviposition behaviour of female mosquitoes and larval sur-

[a]Department of Public Health and Preventive Medicine, Institute of Vector Control and Zoonoses, Hosur–635 126, India.
[b]52, Rashmi Apartments, D'Monte Road, Bandra, Mumbai–400 050, India.

vival, which are influenced by biotic and abiotic environmental factors. In the past three decades there have been changes in the rice cultivation practices following the increase in the irrigation schemes and the area under rice cultivation, and the introduction of high yielding varieties (HYVs) of paddy leading to the green revolution in India. The HYV programme was launched in 1966 in India and the area planted with HYVs increased from 0.9 million ha in 1966 to 26.6 million ha in 1985.[2] It resulted in the expansion of breeding surface for *Culex vishnui* subgroup species, viz. *Cx. tritaeniorhynchus, Cx. pseudovishnui* and *Cx. vishnui*, the most important vectors of Japanese encephalitis (JE) in south India. The increase in the abundance of vectors during this period synchronised with the outbreaks of JE in Tirunelveli in 1978 and subsequently in 1981 and 1983 in South Arcot district of Tamil Nadu.[3]

The changes in agronomical practices in rice fields influence the mosquito breeding and thereby could increase or decrease the disease incidence.[4] The improved varieties of rice have thick and sturdy stems and also have short and erect leaves that permit greater penetration of sunlight than that of in case of traditional varieties, as a result of which the microclimatic conditions of the paddy fields are changed. The degree of shading might determine the suitability of the rice fields for colonisation by different mosquito species and potential predators and the plant canopy may also prevent the oviposition of mosquitoes by acting as a physical barrier.[5]

Although several studies have been conducted on the ecological succession of mosquito species in relation to different stages of plant growth in the rice fields in India,[6] the effect of plant spacing and canopy on the immature population of mosquitoes has not been studied in the rice field planted with HYVs. The present study was conducted to determine the effects of plant spacing and canopy on the population of mosquito immatures in the rice fields.

MATERIALS AND METHODS

The study was conducted in the campus of the Agricultural College and Research Institute, Madurai during 'Kuruvai' crop season from December 1992 to Jaunary 1993, to study the influence of plant spacing on the populations of mosquito immatures and the associated aquatic insect fauna in the rice fields. For this study, three paddy varieties, ADT36, IR50 (short duration variety–110 days) and IR20 (medium duration variety–120 days) were selected. Two types of plant spacing were selected for each paddy variety and six different combinations of rice plots were made as follows in a randomised block design.

T1–ADT36 (80 hills/m^2), T2–IR50 (80 hills/m^2), T3–IR20 (80 hills/m^2), T4–ADT36 (60 hills/m^2), T5–IR50 (60 hills/m^2) and T6–IR20 (60 hills/m^2).

Each rice plot had four replicates of 40 sq m size. At the time of planting, urea (22 kg/acre) and Diammonium phosphate (50 kg/acre) were applied basally. After three weeks, all the fields were top-dressed with fertilizers. Fertilizers are applied in three parts during the growth of paddy. One at the time of planting (basal application),

Table 1. Influence of plant spacing on the population of culicine immatures in rice fields (No./m^2)

Weeks after plant-ation	Normal spacing						Wider spacing					
	T1–ADT36		T2–IR50		T3–IR20		T4–ADT36		T5–IR50		T6–IR20	
	III/IV instar larvae	Pupae	III/IV instar larvae	Pupae	III/IV instar larvae	Pupae	III/IV instar larvae	Pupae	III/IV instar larvae	Pupae	III/IV instar larvae	Pupae
1	10.0	0.0	17.5	0.0	12.5	0.0	5.0	0.0	17.5	0.0	25.0	0.0
2	595.0	47.5	620.0	97.5	795.0	77.5	227.5	27.5	130.0	5.0	207.5	20.0
3	17.5	0.0	35.0	2.5	5.5	0.0	15.0	0.0	5.0	0.0	10.0	0.0
4	22.5	0.0	35.0	15.0	37.5	0.0	15.0	2.5	15.0	0.0	15.0	2.5
5	25.5	0.0	25.0	0.0	15.0	0.0	5.0	0.0	7.5	0.0	2.5	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Analysis of variance

Source	DF	SS		MS		F-value	
		III/IV instar larvae	Pupae	III/IV instar larvae	Pupae	III/IV instar larvae	Pupae
Replicates	3	0.27	0.03	0.09	0.01	1.52	0.58
Factors							
Spacing	1	1.25	0.26	1.25	0.26	21.37*	17.05*
Variety	2	0.04	0.01	0.02	0.01	0.37^{+}	0.29^{+}
Period	5	34.77	6.79	6.95	1.36	118.80*	89.14*
Interaction							
Period x spacing	5	1.42	1.66	0.28	0.33	4.83*	21.84*
Period x variety	10	0.56	0.08	0.06	0.01	0.95^{+}	0.55^{+}
Error	91	5.27	1.37	0.06			

* $p < 0.001$; $^{+}$Not significant.

Factorial randomised block design

$\bar{x}$ log (n + 1)	T1	T2	T3	T4	T5	T6	CD-value
III/IV instar larvae	0.54^{ab}	0.62^{bc}	0.52^{acd}	0.37^{d}	0.36^{d}	0.39^{ad}	0.14
Pupae	0.13^{ab}	0.17^{bc}	0.15^{abc}	0.09^{acd}	0.03^{d}	0.08^{ad}	0.07

Values with similar superscripts do not differ significantly from each other.

second one is first top-dressing (done after three weeks) and third one is second top-dressing (done after five weeks). This is precisely written as all the fields were top-dressed with fertilizers. No pesticide was applied throughout the study. Samples of mosquito immatures were collected using a metal quadrat (0.33 x 0.33 x 15 cm) open at both ends. The quadrat was pushed well into the soil in the rice fields at random sites. The mosquito immatures (III and IV instar larvae and pupae) and other aquatic insects present inside the quadrat were collected by draining the water using an enamel bowl and then counted. After counting the insects separately according to their taxonomic classification, they were released in the same field and then the density of each group of insect was converted to numbers per sq m. The mosquito pupae collected were brought to the laboratory for the species identification after emergence. Samples were taken three times in a week from 1 or 2 days after transplantation. Sampling was continued up to six weeks, since earlier studies,[7] conducted in the rice fields in Madurai district showed that maximum breeding of mosquitoes occurs only during first few weeks after transplantation and there is a natural decline in the population of mosquito immatures from the six weeks onwards.

The height of plants was measured by using a 30 cm scale randomly at five different sites in the rice field and the average values were noted. The plant canopy was estimated by measuring the illumination at the water surface by an illuminometer or lux meter at 1000 hours. A scale of 1 m was thrown into the field randomly at three sites and for every 20 cm along the scale, the readings were noted in lux meter and the average value was calculated for each field.[8] The effect of plant canopy of high yielding paddy varieties on the population of mosquito immatures was compared with that of 'Ponni', a long-term traditional paddy variety (130–140 days) which was cultivated at 40 hills/m^2.

By using the SPSS/PC$^+$ statistical package version 4.0.1 (SPSS, Inc., Chicago, IL, USA, 1984–1990), two-way ANOVA (analysis of variance) was performed on log (n + 1) transformed data to find out the effect of different plant spacings on the population of mosquito immatures and other groups of aquatic insects and then subjected to factorial randomised block design (FRBD) to find out the difference between the plant spacings and also between the paddy varieties.

RESULTS

Effect of plant spacing and paddy varieties

Three paddy varieties and two plant spacings formed six different combinations. Results showed that the fields, T1, T2 and T3 planted with normal spacing, had higher population of culicine (III/IV instar) larvae and pupae than the fields (T4, T5 and T6) with wider spacing (Table 1). ANOVA on log (n + 1) transformed data showed that the plant spacing had a significant effect on the population density of the culicine larvae ($F = 21.37$; $p < 0.001$) and the pupae ($F = 17.05$; $p < 0.001$). However, the plant varieties had no significant effect on the population of culicine immatures. The interaction between the period and the spacing was sig-

Table 2. Influence of plant spacing on the population of anopheline immatures in rice fields (No./m^2)

Weeks after plant-ation	Normal spacing						Wider spacing					
	T1–ADT36		T2–IR50		T3–IR20		T4–ADT36		T5–IR50		T6–IR20	
	III/IV instar larvae	Pupae	III/IV instar larvae	Pupae	III/IV instar larvae	Pupae	III/IV instar larvae	Pupae	III/IV instar larvae	Pupae	III/IV instar larvae	Pupae
1	25.0	0.0	30.0	0.0	35.0	0.0	52.5	0.0	70.0	0.0	42.5	0.0
2	767.5	7.0	1012.5	155.5	757.5	82.5	315.0	37.5	187.5	0.0	230.0	27.5
3	10.0	0.0	30.0	5.0	7.5	0.0	5.0	2.5	5.0	0.0	2.5	0.0
4	20.0	0.0	20.0	0.0	12.5	0.0	10.0	0.0	10.0	0.0	25.0	0.0
5	7.5	0.0	40.0	0.0	7.5	0.0	5.0	0.0	10.0	0.0	15.0	0.0
6	2.5	0.0	10.0	0.0	0.0	0.0	0.0	0.0	5.0	0.0	0.0	0.0

Analysis of variance

Source	DF	SS		MS		F-value	
		III/IV instar larvae	Pupae	III/IV instar larvae	Pupae	III/IV instar larvae	Pupae
Replicates	3	0.76	0.19	0.25	0.06	4.53	3.35
Factors							
Spacing	1	0.59	0.40	0.59	0.40	10.43*	20.78**
Variety	2	0.33	0.03	0.16	0.01	2.89^{+}	0.74^{+}
Period	5	38.57	7.35	7.71	1.47	137.60	76.70**
Interaction							
Period x spacing	5	1.96	1.72	0.39	0.35	6.99**	18.01**
Period x variety	10	0.57	0.35	0.06	0.04	1.01^{+}	1.81^{+}
Error	91	5.05		0.06			

* $p < 0.01$; ** $p < 0.001$; $^{+}$Not significant.

Factorial randomised block design

$\sqrt{x}$ log (n + 1)	T1	T2	T3	T4	T5	T6	CD-value
III/IV instar larvae	0.55^{ab}	0.72^{b}	0.52^{a}	0.45^{a}	0.48^{a}	0.47^{a}	0.14
Pupae	0.15^{ab}	0.18^{ab}	0.16^{a}	0.10^{acd}	0.0^{c}	0.07^{ac}	0.08

nificant, since the population of culicine larvae and pupae decreased from third week onwards in all the rice plots. But the interaction between the periods and varieties was not significant. FRBD analysis showed that with ADT36 and IR50 varieties the population of culicine (III/IV instar) larvae in the two types of plant spacing differed significantly, but there was no significant difference for IR20. For IR50, the population of culicine pupae was significantly different between two types of plant spacing, however, for the other paddy varieties, there was no significant difference between the two spacings.

The population of anopheline immatures was also higher in the rice plots with normal spacing than those with wider spacing (Table 2). ANOVA on log (n + 1) transformed data of anopheline (III/IV instar) larvae and pupae showed that the plant spacing had a significant effect on the population of anopheline immatures, but not the paddy varieties. A significant interaction was observed between the spacing and the period, since the populations of anopheline larvae ($F = 6.99$; $p < 0.001$) and pupae ($F = 18.01$; $p < 0.001$) declined two weeks after transplantation in all the six rice plots. FRBD analysis showed that the population of anopheline larvae and pupae in the field with IR50 vary with the plant spacing, however, with other two paddy varieties they did not differ significantly.

Identification of adult culicine mosquitoes reared from pupae (*Culex tritaeniorhynchus, Cx. pseudovishnui* and *Cx. vishnui*) showed that although higher number of pupae (23, 3 and 64 respectively) were collected in the fields with normally spaced plants than those spaced widely (8, 2 and 12 pupae respectively), there was no statistically significant difference in the species composition of mosquitoes between the fields with different plant spacings and varieties ($\chi^2 = 6.3$; $df = 10$; $p > 0.05$). The anophelines collected during the study period were *Anopheles subpictus* and *An. vagus*. ANOVA showed that the plant spacing had a significant effect on the population of chironomids and libellulids. FRBD analysis showed that the population of chironomids or libellulids was significantly higher with normally spaced ADT36 than those with wider spacing. In fields planted with IR50 and IR20 varieties there was no significant difference in the population of chironomids between the two spacings. The abundance of other insects such as corixids, notonectids, hydrometrids, gerrids, veliids, coengarionids, dytiscids, hydrophilids and ephemeropteran nymphs were not significantly affected by the plant spacing.

Effect of plant canopy

The fields with IR50 variety were studied to measure the effect of plant canopy on the population of mosquito immatures. The development of plant canopy in the rice fields was inversely related to the light intensity at the water surface. Lux meter readings showed that the light intensity decreased as the plant height increased in all the fields (Table 3). In fields with the plant densities of 80 hills/m^2 and 60 hills/m^2, the light intensity remained above 50,000 lux for two weeks after transplantation but, after six weeks it declined to 17,500 lux.

Table 3. Comparison of density of mosquito immatures (No./m^2) and plant densities in rice fields

Weeks after plantation	Plant variety/ density														
	IR50 (80 hills/m^2)					IR50 (60 hills/m^2)					Ponni (40 hills/m^2)				
	Average light intensity (lux)	Culicines		Anophelines		Average light intensity (lux)	Culicines		Anophelines		Average light intensity (lux)	Culicines		Anophelines	
		III/IV instar larvae	Pupae	III/IV instar larvae	Pupae		III/IV instar larvae	Pupae	III/IV instar larvae	Pupae		III/IV instar larvae	Pupae	III/IV instar larvae	Pupae
1	50,000+	17.5	0	3	0	50,000 +	17.5	0	70	0	50,000+	55.6	0	58.9	0
2	50,000+	620	97.5	1012.5	155	50,000 +	130.5	5	187.5	0	50,000+	358.9	110	254.4	40
3	48,000	35	2.5	30	5	48,300	5	0	5	0	50,000+	234.4	45.6	23.3	1.1
4	33,300	35	0	20	0	37,600	15	0	10	0	22,700	272.2	14.4	73.3	1.1
5	18,000	25	0	40	0	23,800	7.5	0	10	0	18,200	70	7.8	23.3	0
6	17,500	0	0	10	0	20,200	0	0	5	0	14,500	5.6	0	5.6	0

+above 50,000 lux.

Although the light intensity was above 50,000 lux in fields with both plant densities and at two weeks after transplantation, the population of late larval instars and the pupae of both anophelines and culicines were higher in the fields with dense plants (80 hills/m^2) than those with sparsely planted rice (60 hills/m^2), and thereafter they declined in both type of fields (Table 3). These differences were significant on ANOVA performed on log (n+1) transformed data ($F = 11.64$; $p < 0.05$ and $F = 15.52$; $p < 0.05$ for the larvae and the pupae of culicines respectively; and $F = 8.82$; $p < 0.05$ and $F = 11.18$; $p < 0.05$ for the larvae and the pupae of anophelines respectively).

In other plots in which 'Ponni', a long-term traditional paddy variety (130–140 days) was cultivated at 40 hills/m^2, the light intensity remained above 50,000 lux for three weeks and then declined. However, the population of culicine (III/IV instar) larvae was high at four weeks after transplantation when the light intensity was only 22,700 lux (Table 3). The population of anopheline (III/IV instar) larvae was high at two weeks after transplantation when the light intensity was above 50,000 lux and thereafter the population declined. These results suggested that plant canopy did not inhibit the oviposition of female culicines, but it could be responsible for the decline in anopheline population in later stages of plant growth.

DISCUSSION

Agricultural practices in rice fields can influence the level of mosquito production and potential for mosquito-borne diseases. The use of fertilisers, pesticides, type of tillage, type of irrigation and cropping systems determine the dynamics and abundance of biotic community in the rice fields.[9] The present study showed that the fields with dense rice planting had higher population of mosquito immatures than those with sparsely spaced plants. Fields with dense plants might be more attractive to female mosquitoes for oviposition during the first few weeks after transplantation. Moreover, the dense vegetation could be more favourable for the larvae to escape from the predators. These results are in conformity with the results of Miura *et al*,[1] although they adopted a different methodology—taking samples of immatures of *Cx. tarsalis* in the same field with dense and sparse rice plant stands at different sites. In contrast, Freeborn[10] collected larger number of anopheline larvae in sparsely planted rice stands than in those with dense rice stands. This reveals that different species of mosquitoes may have different ovipositional behaviour.

Collins and Washino[11] pointed out that because of the inability of ovipositing females to penetrate dense vegetation, there was a remarkable decline in the population of *Cx. tarsalis* larvae in the interior of the rice fields. Similarly, in south India, Russell and Rao[12] found that *An. culicifacies* ceased to breed in the rice fields due to the mechanical obstruction of its oviposition when the plant canopy became thick. In the present study, although the light intensity was comparable (above 50,000 lux) in the fields with two different plant densities at two weeks after transplantation, the population of larvae and pupae of anophelines and culicines were significantly higher in the fields with densely spaced

plants than those with sparsely planted rice plants and thereafter the population declined in both the fields. This suggests that the plant canopy did not inhibit oviposition of female mosquitoes in the early stages of paddy growth, but it was responsible for the decline in the population in the later stages of paddy growth. Similarly, Kramer[8] found that the population of *Cx. tarsalis* larvae increased as the plant height increased in the earlier phase of paddy growth, whereas in the later stage opposite trend occurred in the rice fields in California. The plant spacing did not affect the population of other aquatic insects except chironomids and libellulids and this may be due to colonisation of these insects during the later stages of plant growth.

ACKNOWLEDGEMENTS

We thank Dr. A. Gajanana, Officer-in-Charge, Centre for Research in Medical Entomology (ICMR), Madurai, for providing facilities and to Professor Dr. M.S. Venugopal, Deptt. of Entomology, Agricultural College and Research Institute (TNAU), Madurai for his valuable help in conducting field experiments in the Agricultural College campus. We also acknowledge the technical assistance rendered by the staff of control section, CRME, in field collections throughout the study and the financial assistance by CSIR.

REFERENCES

1. Miura, T., R.M. Takahashi and W.H. Wilder (1983). Spatial distribution of *Culex tarsalis* larvae within rice paddies. *Proc. Calif. Mosq. Vec. Contr. Assoc.*, **51:** 58–60.
2. Anon. (1985). *Fertiliser Statistics for 1984–1985* (Fertiliser Association of India, New Delhi).
3. Victor, T.J. and R. Reuben (2000). Effects of organic and inorganic fertilisers on mosquito populations in rice fields of southern India. *Med. Vet. Entomol.*, **14:** 361–368.
4. Mogi, M. and I. Miyagi (1990). Colonisation of rice fields by mosquitoes (Diptera: Culicidae) and larvivorous predators in asynchronous rice cultivation areas in the Philippines. *J. Med. Entomol.*, **27:** 530–536.
5. Mather, T.J. and T.T. That (1984). Environmental management for vector control in rice fields (Food and Agricultural Organisatin, Rome), FAO Irrigation Drain Paper, **41:** 152.
6. Reuben, R. (1971). Studies on the mosquitoes of North Arcot District, Madras State, India, Pt V, Breeding places of the *Culex vishnui* group of species. *J. Med. Entomol.*, **80:** 363–366.
7. Rajendran, R. and R. Reuben (1991). Evaluation of waterfern *Azolla microphylla* for mosquito management in rice land agro–ecosystem of south India. *Med. Vet. Entomol.*, **5:** 299–310.
8. Kramer, V.L. (1988). The ecology and biological control of mosquitoes in California wild and white rice fields (Ph.D. Thesis; University of California, Berkeley).
9. Lacey, L.A. and C.M. Lacey (1990). The medical importance of rice land mosquitoes and their control using alternatives to chemical insecticides. *J. Amer. Mosq. Contr. Assoc.*, **6:** 1–93.
10. Freeborn, S.B. (1917). The malaria problem in rice fields. *Calif. State J. Med.*, (Oct.): 1–6.
11. Collins, F.H. and R.K. Washino (1980). The effects of irrigation water source and crop rotation on the abundance of *Culex tarsalis* in California rice fields. *Proc. Calif. Mosq. Vec. Contr. Assoc.*, **48:** 103–108.
12. Russell, P.F. and T.R. Rao (1942). On relation of mechanical obstruction and shade to oviposition of *Anopheles culicifacies*. *J. Exp. Zool.*, **91:** 303–329.

Indian Journal of Malariology
Vol. 37, March–June 2000, pp. 27–33.

Tumor Necrosis Factor-Alpha in Patients with Malaria

SARMAN SINGH[a], NITI SINGH[a] and ROHINI HANDA[a]

Serum concentration of Tumor Necrosis Factor-alpha (TNF-α) was observed in 54 parasitologically confirmed cases of malaria. Of them, 15 cases were *Plasmodium falciparum* with cerebral involvement, three cases with mixed infections of *P. falciparum* and *P. vivax*, 32 cases of *P. vivax*, three cases of *P. malariae* and one case of *P. ovale*. Five out of 15 patients of *P. falciparum* (33.3 per cent), one out of 54 patients with mixed infection of *P. falciparum* and *P. malariae* (1.8 per cent) and the sole case of *P. ovale* (1.8 per cent) had fatal outcome. The serum TNF-α measured by avidin-biotin sandwich ELISA, was found to be significantly raised in *P. falciparum* and more so in fatal infections. The degree of parasitaemia, due to single or double infection, had positive effect on cytokine production. The mean TNF-α concentration was statistically significantly higher ($p < 0.001$) in *P. falciparum* than in *P. vivax* parasites infection. The mean TNF-α values in *P. falciparum* and *P. vivax* were 915 and 280.6 against the values in normal healthy controls of 12.9 pcg/ml respectively ($p < 0.001$). The study thus showed that the serum concentration of TNF-α correlated well with severity of malaria and these values could be used as an important prognostic marker of the disease.

Keywords: Cerebral malaria, Falciparum malaria, India, Malaria patients, Tumor necrosis factor-alpha

INTRODUCTION

Although malaria has disappeared from many countries including India, mainly as a result of socio-economic improvement and successful antimalarial campaigns in these countries, over 2000 million (41 per cent world's population) people still remain exposed to this disease and mortality is quite high in case of cerebral (falciparum) malaria.[1,2] Acquired immunity

[a]Department of Laboratory Medicine, Clinical Microbiology Division, All India Institute of Medical Sciences, Ansari Nagar, New Delhi–110 029, India.

to malaria does occur, but it requires repeated exposures to the parasite. This fact holds true, when one finds that mortality rate even with high *P. falciparum* parasitaemia is lowest in hyperendemic adult population.[1] Several mechanisms are involved in non-specific as well as specific protection. The accumulated evidences suggest that specific humoral immunity is Th-cell dependent and involves production of various cytokines by activated CD4+ cell population.[1] Tumor necrosis factor-alpha (TNF-α) or cachectin is a cytokine which is produced in response to malarial antigen and acts on various populations of the nucleated cells.[3,4] Its role in several parasitic diseases such as malaria, leishmaniasis and toxoplasmosis has been documented.[4-7] Conflicting reports have appeared about its role in malaria disease. Some studies have reported increased levels of TNF as protective, while others have shown TNF production responsible for the development of clinical symptoms and complications like disseminated intravascular coagulation (DIC), hypotension and hypoglycemia and the rest, in cerebral malaria.[1,2,8] However, no study has been reported from India. Here, we report our preliminary work on TNF-α concentration in malaria and its correlation with severity of the disease.

MATERIALS AND METHODS

In this study, 3000 patients from outdoor and indoor departments of All India Institute of Medical Sciences (AIIMS), New Delhi were screened for various haemoparasitic infections during 1992–94 and during a moderate malaria outbreak in Delhi during 1996–97. Out of these, serum samples from the patients with diagnosis of falciparum malaria with or without cerebral involvement was established in 15, vivax malaria in 32, double infection of *P. falciparum* + *P. vivax* in three cases and three cases of *P. malariae* (including one case of *P. falciparum* and *P. malariae* mixed infection) were collected. Serum sample from one patient with diagnosis of *P. ovale* infection, established on the morphological basis, was also collected. This patient came from Delhi only and never travelled abroad.[9] Twenty apparently healthy persons with no clinico-parasitological findings were included as negative controls. To determine the parasite load and serum TNF-α values, the clinical samples were collected daily till cure or death of the patient in cases of falciparum malaria. Other samples were collected only once at the time of hospitalization. First serum sample was collected before starting the specific treatment.

Diagnosis of malaria and parasite count

For the diagnosis of malaria thin and thick blood smears were made and stained with Leishman. Thin smear negative samples, were concentrated (dehaemoglobinized) and re-examined.[10] The parasite count was done on all positive smears following standard method. The asexual forms of the parasite were counted against 10 white blood cells and the number was multiplied with 800, which corresponds to 8000 WBC (or parasites) per microlitre. Cerebral malaria was diagnosed when *P. falciparum* positive cases showed signs of cerebral involvement.

Serum samples

Five millilitre venous blood was collected in a

plain vial from all positive patients, serum was separated and stored at –20°C till further use. Sera from 20 years age and sex matched healthy controls were also collected simultaneously. Whenever possible, repeat serum samples were collected every day from proven cerebral malaria cases.

TNF-alpha estimation

All serum samples were centrifuged and only clear supernatants were tested for human TNF-a. For TNF-a quantitative estimation, a ready-to-use enzyme immunoassay (EIA) kit (Boehringer–Mannheim Biochemica, Germany) was used. The whole process was undertaken at room temperature (approx 25°C). Briefly, twenty microlitres of sample/standard was pipetted into the microwells, precoated with two anti-TNF-a monoclonal biotinylated antibodies. To each well 200 ml of immuno-reagent was added. This reagent was prepared by adding 0.05 ml of anti-TNF-a HRP to 0.9 ml of incubation buffer and 0.05 ml of anti-TNF-a-biotin. After putting the immuno-reagent, plate was covered and incubated for four hour at room temperature with regular low speed shaking (Anthos-HT2) followed by three washings (Anthos-Autowash). 200 ml of TMB substrate solution was added to each well, plate was covered and incubated again for 25 min at room temperature with constant shaking. The reaction was stopped with 1M H_2SO_4. The optical density (OD) reading was taken at 450 nm (ref. filter 690 nm) by Anthos-HT2 ELISA reader. The quantitative estimation of human TNF-a in these samples was made by calibrating a curve drawn from the known standards (provided) on the reader and the values in picograms (pcg/ml) were obtained from this curve. The test was run in duplicate.

RESULTS

Out of 3000 cases suspected to have malaria 120 had *P. vivax*, 90 *P. falciparum*, three *P. malariae* (Fig. 1) and one *P. ovale*. Four patients had mixed infection of *P. vivax* and *P. falciparum*, 37 patients had mixed infection of *P. falciparum* and *P. malariae*. Five of the 15 (33.3 per cent) patients with falciparum malaria had fatal outcome. Both these fatal cases have already been reported elsewhere.[9,11]

Out of these 218 confirmed patients of malaria only 54 were investigated for serum TNF-a values in 1996-97. The TNF-a mean values in normal Indian subjects were 12.9 pcg/ml. The values were significantly ($p < 0.001$) higher in the patients infected with *P. falciparum* (80–5968 pcg/ml) than *P. vivax* (8–1880 pcg/ml) (Table 1). Our observations on severe cases of malaria showed that TNF-a values correlated directly with parasite load (Fig. 1). The high TNF-a concentration directly also correlated with bad or poor prognosis and decreasing values indicated recovery from the disease. However, sudden fall in serum TNF-a values indicated worst prognosis or fatal outcome. In two fatal cases of falciparum malaria, the parasitaemia was found as high as 11,28,000/ml (Fig. 2) and the mean TNF-a concentration in these cases was 2616 pcg/ml at the time of their hospitalization. One patient died of DIC and hypoglycemic coma within six hours and the repeat TNF-a values could not be determined,

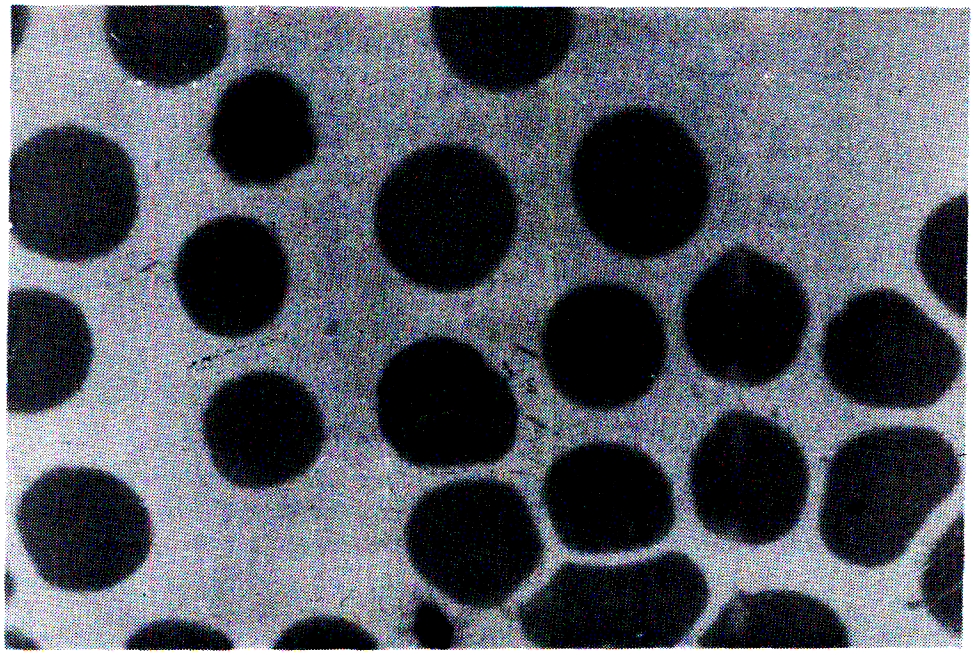

Fig. 1: The peripheral blood smear stained with Giemsa stain. Note band shaped ring trophozoites of *Plasmodium malariae* (x1000)

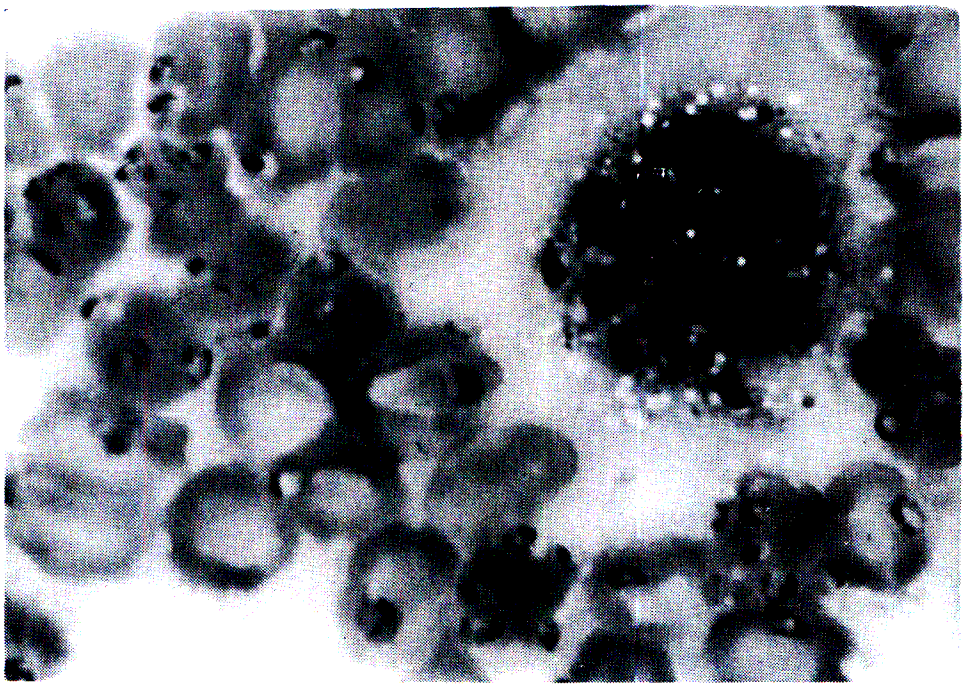

Fig. 2: The peripheral blood smear stained with Giemsa stain from a fatal case of falciparum malaria. Note the severity of parasitaemia and parasitophagus monocyte (x1000)

Table 1. Serum TNF-α values (in picograms/ml) infection

Patient group	No.	TNF-α	
		Range	Mean
P. vivax	32	8-1880	280.6
P. falciparum	15	80-5968	915.0
P. falciparum + *P. vivax*	3	298-5968	1114.6
P. malariae	3	240-2984	3104.0
P. ovale (1)	1	1740	1740.0
Healthy control	20	0-60	12.9
Total	74		

The difference in serum TNF-α values were statistically significant among the healthy controls vs. malaria infected and cerebral malaria vs. benign malaria patients.

while other patient died next day. His serum TNF-α values at the terminal stage were found undetectable. In other three fatal cases of falciparum malaria serum concentrations of TNF-α were more than 700 pcg/ml. The mean TNF-α concentration in the patients with double infection was also found very high (Table 1). The TNF-α concentration of serum TNF-α fell down with clearance of the parasitaemia after treatment. However, in two patients TNF-α values increased after treatment. One of these patients developed resistant and the second had DIC at the time of first and second sampling, but recovered at the time of third sampling.

DISCUSSION

The relationship between parasitaemia and mortality in falciparum malaria was first described in 1937 by Field and Niven working in Kuala Lumpur.[1] They noted that parasitaemias over 100,000/μl (approx 2–3 per cent) were correlated with increased mortality and that half of their patients with parasitaemia more than 500,000/μl died. Since then parasitaemia is being used as an index of severity. However, in a fair number of terminally ill patients, malaria parasites may not be detectable by peripheral blood smear examinations, particularly when it is complicated by DIC.[1-3] Thus, additional or alternative diagnostic and prognostic parameters are always welcome.

Present study indicates that one such parameter could be the tumor necrosis factor alpha. TNF-α or cachectin is one principal cytokine produced early in response to infection.[4] This is also a mediator of general inflammation and is responsible for, together with interleukin-1 (IL-1), fever through the stimulation of hypothalamic prostaglandin–E2.[1-4] Its effects on host have been reported both beneficial and harmful which is probably dose dependent.[2] In the literature, increased TNF-α production in falciparum malaria, has been reported from Gambia, Malawi, the Solomon Islands and Europe but not from any southeast Asian countries, which is an another falciparum malaria endemic subcontinent. In these studies, it has been reported that TNF-α concentrations of more than 100 pcg/ml are highly associated with cerebral involvement and death of the patient.[2] Our observations also verify these findings. In our study, falciparum malaria cases with more than 700 pcg/ml of serum TNF-α invariably had fatal outcome. The sole case of *P. ovale* was diagnosed in a 60 years old Sikh woman, who

resided in Delhi for the last 30 years and had never travelled abroad. She was hospitalized in comatosed state at AIIMS and died within 48 hours in spite of starting quinine sulphate infusion. Her TNF-α concentration was 1740 pcg/ml. An other fatal case was of mixed infection of *P. falciparum* and *P. malariae*. This was a young pregnant medical doctor from Bihar who never travelled abroad and was residing in Delhi for the last three years. Though her *P. malariae* got cleared with quinine therapy but she and her fetus both succumbed to falciparum infection in spite of best possible medical care and treatment.[11] Her serum TNF-α values were found to be very high (5968 pcg/ml).

Rise in TNF-α values during second and third sampling in one patient who was hospitalized with DIC and recovered later with antimalarial treatment indicates that during DIC and severe hypoglycemia, the malarial parasites are not accessible to the immune system due to their scanty presence in the peripheral blood. These parasites sequester knob proteins through the infected red blood cells, and cytoadhere to the cerebral and other vasculature. Since, interleukin-1 and interferon-gamma were not measured in these cases, which are essential for the production of TNF-α from the activated macrophages, it is also likely that one or more of these mediators did not work synergistically during severe DIC.[3,4,8]

Pathogenesis of falciparum malaria has been studied to the great extent in recent years.[12-15] Inter- and intra-species differences in erythrocyte rosette formation have been documented. The falciparum species shows adherence of parasitised red blood cells to the vasculature endothelium, which is due to the presence of knob associated protein on the parasites and it is responsible for DIC and fatal hypotension. Absence of knob associated protein in *P. vivax* could probably explain the failure of production by this species. However, not much is published on *P. ovale* and *P. malariae* that are conventionally considered benign infection. The study revealed that more cytokine work is required on these species of Plasmodium. Thus it is concluded that TNF-α can be used as an alternative marker of disease prognosis in severe malaria.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the technical assistance of Mr. Jugesh Kumar and Ms. Veena Kumari, both from the Department of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi.

REFERENCES

1. Carlson, J. (1993). Erythrocyte reosetting in *Plasmodium falciparum* malaria: With special reference to the pathogenesis of cerebral malaria. *Scand. J. Infect. Dis.*, **86**: 1–79.
2. Clark, L.A., G. Chaudhri and W.B. Cowden (1989). Roles of tumor necrosis factor in the illness and pathology of malaria. *Trans. R. Soc. Trop. Med. Hyg.*, **83**: 436–440.
3. Clark, L.A., K.A. Rockett and W.B. Cowden (1992). Tumour necrosis factor. *Tumour Necrosis Factor*, ed. B. Beutler (Raven Press, Ltd., New York): 303–328.
4. Silamut, K. and N.J. White (1993). Relation of the stage of parasite development in the peripheral

blood to prognosis in severe falciparum malaria. *Trans. R. Soc. Trop. Med. Hyg.*, **87:** 436–443.

5. Beutler, B.A., I.W. Milsark and A. Cerami (1985). Cachectin-tumour necrosis factor production, distribution and metabolic fate *in vivo*. *J. Immune*, **135:** 3972–3977.
6. Sypek, J.P. and D.J. Wyler (1991). Anti-leishmanial defense in macrophages triggered by tumour necrosis factor expressed on CD4+ T-lymphocyte plasma membrane. *J. Exp. Med.*, **174:** 755–759.
7. Beaman, M.H. and J.S. Wong Sin-Yew Remington (1992). Cytokines, toxoplasma and intracellular parasitism. *Immunol. Dev.*, **127:** 97–117.
8. Bate, C.A.W., J. Taveme, N.D. Karunaweera. K.N. Mendis, D. Kwiatkowski and J.H.L. Playfair (1992). Serological relationship of tumor necrosis factor-inducing exoantigens *Plasmodium falciparum* and *Plasmodium vivax*. *Infect. Immunity*, **60:** 1241–1243.
9. Singh, S. (1999). *Plasmodium ovale* infection in Delhi. *Indian J. Paed.*, **66**(3): 460.
10. Singh, S. and J.C. Samantaray (1989). Blood-films in the diagnosis of malaria. *Lancet*, **II:** 1165–1166.
11. Singh, S., R. Guleria and S. Kumar (1999). Multidrug resistant fatal falciparum malaria in a pregnant women. *J. Assoc. Phyic. India*, **47:** 346.
12. Carlson, J., H.P. Ekre, H. Helmby, J. Gysin, B. Greenwood and M.Wahlgren (1992). Distribution of *Plasmodium falciparum* erythrocyte rosettes by standard heaprin and heparin devoid of anti-coagulant activity. *American J. Trop. Med. Hyg.*, **46:** 595–602.
13. Udomasantetch, R., B.Wahlin, J. Carlson, K. Berzins, M. Torii, M. Aikawa, P. Perlmann and M. Wahlgren (1989). *Plasmodium falciparum* infected erythrocytes form spontaneous erythrocyte rosettes. *J. Exp. Med.*, **169:** 1835–1840.
14. Grau, G.E., G. Bjeler and P. Pontaire (1990). Significance of cytokine production and adhesion molecules in malarial immunopathology. *Immunol. Lett.*, **25:** 189–194.
15. Kremsner, P.G., H. Grundmann, S. Neifer, K. Silwa, G. Sahlmuler, B. Hegenschied and U. Bienzle (1991). Pentoxyfylline prevents murine cerebral malaria. *J. Infect. Dis.*, **164:** 605–608.

Short Notes

Indian Journal of Malariology
Vol. 37, March–June 2000, pp. 34–38.

Prevalence and Association of Sickle-cell Haemoglobin and *P. falciparum* Infection among Tribals in Koraput District, Orissa State

L.K. DAS[a]

Keywords: Malaria, *P. falciparum*, HbS

The interaction between sickle-cell haemoglobin (HbS) and malaria (*P. falciparum*) seems to be a well-established phenomenon among the African population.[1] It has been shown that the heterozygotes (HbAS) are less susceptible to falciparum malaria than normal individuals and the gene frequency of HbS is determined by falciparum malaria. Allison presented evidence for lower *P. falciparum* parasitaemia in children with HbAS.[1]

In India, HbS is widely distributed, more common in the upper and scheduled castes than in the tribal groups.[2] Though many studies were carried out on the HbS prevalence,[3-5] the association between HbS and malaria prevalence was studied by a few[6,7] and the observations were inconclusive. Therefore, a study was carried out to investigate the association between sickle-cell haemoglobin and malaria prevalence in a tribal area of Koraput district,

[a]Vector Control Research Centre (ICMR), Medical Complex, Indira Nagar, Pondicherry–605 006, India.

a highly endemic area for *P. falciparum* malaria in Orissa.[8]

Two-stage sampling was adopted to investigate the sickle-cell haemoglobin in the population. In the first stage, 81 villages were randomly selected from 17 Primary Health Centres (PHCs) of erstwhile Koraput district (17°50' and 20°30'N and 81°27' and 84°10'E). Sample size was determined from each village according to the population of the villages by adopting the simple random sampling procedure so as to cover a minimum of 10 per cent of the population. The topography and other details of the district have been described elsewhere.[8] The finger prick blood slides were stained with Giemsa solution. Parasite count was graded according to Bruce-Chwatt[9] and the sickling test was performed following the method of Dacie and Lewis.[10] The data were analysed to examine the variations in the HbS frequency between villages having different levels of malaria prevalence. The distribution of malaria positives and parasitaemia levels among sickle-cell positives and normals were compared using chi-square test.

The overall prevalence rate of sickle-cell trait ranged from 0 to 43.56 per cent in the study villages with a mean of 8.3 per cent. In 20 villages no sickle-cell haemoglobin was recorded, whereas in 35 villages it was less than 10 per cent and in the remaining 25 prevalence was more than 10 per cent. The parasite rate varied widely among the villages and ranged from 0 to 84.4 with a mean of 29.6. There was no correlation between the sickle-cell rate and parasite rates in different villages (Fig. 1).

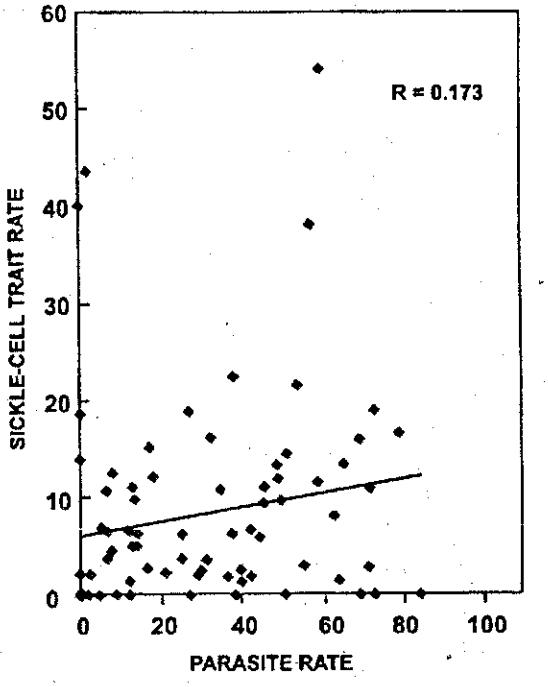

Fig. 1: Scattergram showing village wise parasite and sickle-cell trait rates

Table 1 shows the overall and age-specific parasite rates, sickle-cell rates and parasite rates in sickle-cell positives and negatives. The sickle-cell and parasite rates differ significantly between the age groups ($p < 0.05$). The mean parasite rate in adults (15 years and above) was significantly lower than that of children (1–4 years, $p < 0.05$) but no difference ($p > 0.05$) was observed in the sickle-cell rates between these two groups. The overall odds ratio for the parasite positivity among the sickle-cell positives and normals was 1.06 and is not significant ($p > 0.05$). There was no significant difference ($p > 0.05$) in the frequency of sickle-cell rate between males and females. Similar observation was made when the data were analysed by age groups.

Table 1. Age specific parasite rates among sickle-cell positive and negative individuals

Age group (yrs)	Sickle-cell (+)	Parasite rate	Sickle-cell (–)	Parasite rate
< 1	11	18.18	70	28.57
1–4	72	43.06	800	34.38
5–9	85	37.65	1088	31.62
10–14	33	39.39	480	32.08
15 and above	191	17.28	2610	21.19
Total	392	28.32	5048	26.66

Table 2 shows the parasite rate and sickle-cell rate among different tribal communities. Among the major tribes, sickle-cell rate was significantly lower among the Kondhs and Porjas and it was significantly higher among the Koyas, Alameks and Doras.

Table 3 shows the age-wise comparison of parasite density among the sickle-cell positives and negatives. Only 4+ density was higher among the sickle-cell positives when compared to sickle-cell negatives in all the age groups.

Table 2. Sickle-cell and parasite rates among different tribal communities

Caste	No. examined	SCR	SPR
Major Tribes			
Kondha Dora	191	0.52	1.05
Kondho	339	2.36	32.45
Dhurva	166	2.41	54.22
Porja	440	2.95	15.23
Gadva	278	4.32	17.99
Paiko	96	5.21	10.42
Rana	259	6.95	26.64
Pujari	27	7.41	18.52
Bumia	233	8.58	18.45
Koya	1889	8.89	43.57
Alamek	122	14.75	9.84
Dora	22	18.18	40.91
Others	270	21.48	17.41
Unclassified	142	11.27	18.31
Settlers	359	3.06	7.24
Non-tribals	190	11.58	12.63
SC	446	2.91	11.21

SCR — Sickle-cell rate; SPR — Slide positivity rate for *P. falciparum;* SC — Scheduled castes.

Table 3. Parasite density grades among sicklers and non-sicklers for different age groups

Age group (yrs)	Sickle-cell (+)				Sickle-cell (–)			
	1+	2+	3+	4+	1+	2+	3+	4+
0–1	1	0	1	0	4	5	11	1
1–5	5	10	11	5	80	90	86	21
5–10	12	11	8	1	114	134	84	14
10–15	4	5	3	1	64	61	24	7
≥ 15	19	10	3	1	303	155	80	14
Total	41	36	26	8	565	445	285	57

Earlier, Indian studies have shown that the frequency of sickle-cell trait varied from 5 to 30 per cent[11] and in the Koraput district from 3 to 41 per cent.[3] A study in Orissa, showed that sickle-cell trait was higher in the hilly districts of Orissa when compared to the coastal areas and no significant difference was found in the distribution between genders, though such difference was observed in other studies.[7] Available data on the distribution on HbS indicate that this trait is relatively high among the tribals throughout India except in northwest and extreme south.[11-13] However, a study on the outdoor hospital patients in Orissa showed that the distribution of sickle-cell trait was more common in the upper and scheduled castes than in the tribal groups.[2] Incidence of sickle-cell trait in different tribes in Koraput district was considered by Roy and Roy Chaudhuri[14] but was unable to comment anything due to small sample size.

In the present study, the frequency of sickle-cell trait varied widely among tribal and non-tribal groups. While it is difficult to make any definite conclusion with these varying results, it is obvious that the populations with high frequency of sickle-cell trait were from areas which had a history of being stable malarious that could be comparable to the situation in Africa. In recent years, because of large-scale ecological changes, the endemicity levels varied widely in different villages[15] and there was no correlation between the sickle-cell and malaria parasite rates. There were only a few studies in India on the association of sickle-cell trait and malaria. Desmukh and Sharma[6] presented a positive correlation between the sickle-cell trait and malaria endemicity in Maharashtra. The observation was, however, based on 100 samples correlated with the endemicity levels in five districts. Pant *et al.*[7] reported that no falciparum cases were detected in the sickle-cell positive samples in Gujarat. In this study, no definite correlation could be observed between parasite rates in sickle-cell positives and negatives. A study in Zimbabwe showed that there was no significant difference in the haemoglobin genotype distribution between malarious and non-malarious areas and also between the ethnic regions.[16-18] Frequency of sickle-cell trait in adults in malarious areas is expected to be significantly higher than in children. It has been reported that the presence of sickle-cell trait does not reduce the susceptibility of the individuals to infection but reduces the severity. Children who had died of malaria or who had parasitaemia greater than, 1,00,000/mm^3 rarely included with sickle-cell trait.[1] From the earlier studies and the present study it is clear that the sickle-cell trait does not offer complete protection from *P. falciparum* and even high grade parasitaemia were prevalent among the sickle-cell positives. These observations, however, have some limitations in the absence of actual quantification of parasitaemia and longitudinal follow-up.

REFERENCES

1. Allison, A.C. (1954). The distribution of the sickle-cell trait in East Africa and elsewhere and its apparent relationship to the incidence of subtertian malaria. *Trans. R. Soc. Trop. Med. Hyg.*, **48**(4): 312–318.
2. Kar, B.C., S. Devi and K.C. Dash (1987). The sickle-cell gene is wide spread in India. *Trans. R.*

Soc. Trop. Med. Hyg., **81:** 273–275.

3. Das,. S. R., D.P. Mukharjee and D.B. Sastry (1967). Sickle-cell trait in Koraput district and other parts of India. *Acta Genet. Stat. Med.*, **17:** 62.
4. Ahmed, S.H. (1970). ABO blood groups and sickle-cell trait among the Kondha and Nuka Doras of Koraput district. *Bio-anthropological Research in India*, ed. H.K. Rakshit (Anthropological Survey of India, Calcutta): 75.
5. Sharma, J.C., K. Sharma and R.S. Mohan (1985). A study of some genetic traits in the Khond tribes of Orissa. *J. Indian Anth. Soc.*, **20:** 50–58.
6. Desmukh, V.V. and K.D. Sharma (1968). Deficiency of erythrocyte G-6-PD and sickle-cell trait — A survey of Mahar students at Aurangabad, Maharashtra. *Indian J. Med. Res.*, **56:** 821–825.
7. Pant, C.S., D.K. Gupta, R.M. Bhatt, A.S. Gautam and R.C. Sharma (1992). An epidemiological study of G-6-PD deficiency, sickle-cell haemoglobin and ABO blood groups in relation to malaria incidence in Muslim and Christian communities of Kheda, Gujarat (India). *J. Com. Dis.*, **24**(4): 199–205.
8. Rajagopalan, P.K., P.K. Das, S.P. Pani, P. Jambulingam, S.S.S. Mohapatra, K. Gunasekaran and L.K. Das (1990). Parasitological aspect of malaria persistence in Koraput district, Orissa state, India. *Indian J. Med. Res.*, **91**(A): 44–51.
9. Bruce-Chwatt, L.J. (1985). *Essential Malraiology* (William Heinman Medical Books Ltd. London): 156–452.
10. Dacie, J.V. and S.M. Lewis (1982). *Practical Haemotolgoy* (ELBS, Churchill Livingstone, London): 238.
11. Sharma, Archana (1983). Haemoglobinopathies in India: Some genetical aspects (XV International Congress of Genetics, New Delhi): 35.
12. Chatterjee, J.B. (1966). Haemoglobinopathies, Glucose-6-phosphate dehydrogenase deficiency and allied problems in the Indian subcontinent. *Bull. WHO*, **35:** 837–856.
13. Joshi, Hema, Sarala K. Subbarao and V.P. Sharma (1999). A study of genetic markers in Mewat region, Gurgaon, Haryana. *Indian J. Malariol.*, **36:** 85–89.
14. Roy, D.N. and S.K. Roy Chaudhuri (1967). Sickle-cell trait in the tribal population in Madhya Pradesh and Orissa (India). *J. Indian Med. Assoc.*, **49:** 107–112.
15. Jambulingam, P., S.S.S. Mohapatra, P. Govardhini, Lalit Kumar Das, A. Manoharan, S.P. Pani and P. K. Das (1989). Microlevel epidemiological variations in malaria and its implications on control strategy. *Indian J. Med. Res.*, **93**(A): 371–378.
16. Adewuyi, J.O. and C. Gwanzura (1994). Frequency of the sickle haemoglobin gene in Zimbabwe. *East African Med. J.*, **71**(3): 200–206.
17. Joshi, Hema, K. Raghavendra, Sarala K. Subbarao and V.P. Sharma (1987). Genetic markers in malaria patients in Delhi. *Indian J. Malariol.*, **24:** 33–38.
18. Joshi, Hema, M.S. Malhotra, K. Raghavendra, Sarala K. Subbarao and V.P. Sharma (1998). Genetic studies among Buksa tribals. *J. Parasit. Dis.*, **22:** 136–139.

Indian Journal of Malariology
Vol. 37, March–June 2000, pp. 39–42.

Chloroquine Resistant *Plasmodium falciparum* in Migrant Population

H.C. SRIVASTAVA[a] and S.K. SHARMA[b]

Keywords: Chloroquine, *Plasmodium falciparum*, Resistance

Resistance in *Plasmodium falciparum* to chloroquine and other antimalarials remains a major problem in malaria control now-a-days. Spread of chloroquine resistant *P. falciparum* (CRPF) cases depends on various epidemiological factors. The population migration and easy availability of drugs are also the factors responsible for spreading resistant parasites. In Gujarat, CRPF was first detected from Surat district in 1984.[1] Subsequently varied levels of CRPF cases were detected from different districts.[2,3] The six districts of Gujarat state—Surat, Panchmahals, Bharuch, Baroda, Kheda and Valsad contribute 60 and 73 per cent of total malaria and *P. falciparum* cases respectively. During the past decade, there has been rapid industrialization, urbanization and lot of construction activities in southern districts of Gujarat. As a result, large migratory populations from the endemic area of Gujarat and other states have aggregated in foresaid districts at construction sites, in urban areas and at nearby stone quarries. The present study was carried out to know the magnitude of chloroquine resistant *P. falciparum* in three different areas of Gujarat—Surat City, Sardar Sarovar Narmada (SSN) dam site at Bharuch and Nes PHC of the District Kheda where most of the people, have congregated from different parts of India and substantial increase in *P. falciparum* cases has been observed in spite of regular malaria control measures.

Patients positive for *P. falciparum* (ring stages) were screened through mass blood surveys in slum areas of Surat City, labour settlements at Sardar Sarovar Narmada (SSN) dam site, Bharuch and stone quarry labourers from villages

[a]Malaria Research Centre (Field Station), Civil Hospital, Nadiad–387 001, India.
[b]Malaria Research Centre (Field Station), N-18, Civil Township, Rourkela–769 004, India.

in Nes PHC of Kheda district during 1994–1995. Patients whose urine was negative for chloroquine by Dill and Glazko's method were selected for the detection of chloroquine resistance. *In vivo* tests were carried out on patients belonging to age group of 10 years and above by Rieckmann's method.[4] Chloroquine was administered @ 10 mg/kg body weight on Day 0 and 1, subsequently @ 5 mg/kg body weight on Day 2. Blood smears of all selected cases were taken on Day 0, 2 and 7 and the parasite density per cubic mm of blood was determined. If patient's blood smear was found negative on Day 2 the results were classified as sensitive (S/RI). The patients whose asexual parasitaemia was less than 25 per cent of the original smear, follow-up smear was taken on Day 7. If the latter was negative for malaria parasite the level of resistance was indicated as RI early/late. If parasitaemia still persisted, the resistance was classified as RII level. In case parasite density determined on Day 2 was more than 25 per cent of that recorded on Day 0, the degree of resistance was considered as RIII level. Such patients were given alternative treatment with a single dose of Indicalfin or Metacalfin (Sulfalene 1500 mg + Pyrimethamine 75 mg, adult dose).

In Surat City the total slum population is around 0.43 million which is about 29 per cent of the total urban population. Most of them have rural origin. Migrants from various states of India have been coming to Surat. The flow of migrants from the districts of Maharashtra and Uttar Pradesh has been more than that of from Orissa, Andhra Pradesh and Punjab states.[5] At SSN dam site at Bharuch, 2670 migrants have settled in nine labour colonies. The flow of migrants from Bihar was highest (35.1 per cent) followed by Uttar Pradesh (23.6 per cent), Madhya Pradesh (12.8 per cent), Rajasthan (6.3 per cent) and Gujarat (4 per cent). In Nes PHC of Kheda district, labourers (population 2500) were coming to work in stone quarries from nearby villages. In all the three study areas, the labour movement and the frequency of visit to their native place was usually once in a year, either before monsoon or in the post-monsoon season.

During mass surveys 2462 blood smears (Nes PHC — 436, SSN dam site — 229 and Surat City — 1797) were collected and examined. The highest slide positivity rate 22.5 per cent (98/436) was found in Nes PHC followed by 18.8 per cent (43/229) in SSN dam site and 12.3 per cent (221/1797) in Surat City. *P. falciparum* infection was predominant and was recorded in high proportion (range 74.4–84.6 per cent). In order to detect chloroquine sensitivity 150 *P. falciparum* cases were followed successfully. Out of which 95 were from the slum area of Surat City, 15 from labourers at SSN dam site and 40 from villages of Nes PHC, Kheda. Out of 150 *P. falciparum* cases, 107 (71.3 per cent) exhibited good response (S/RI) where parasitaemia cleared on Day 2 of post-treatment and did not reappear up to Day 7 (Table 1). Remaining 43 (28.6 per cent) cases showed different levels of resistance. RI-early/late resistant cases were maximum in each area, while RII degree of resistance was minimum. RIII level of resistance was detected in 12 per cent *P. falciparum* cases and all these cases were given alternate drug (Sulfalene-Pyrimethamine) on Day 3. All these were negative on Day 7 and were free from all symptoms. The

Table 1. Chloroquine sensitivity of *P. falciparum* in study areas

Study area	Total cases studied	Sensitive cases	Degree of resistance			Total resistance
			RI	RII	RIII	
Surat City	95	70 (73.7)	1 (14.7)	1 (1.0)	10 (10.5)	25 (26.2)
SSN dam site, Bharuch	15	10 (66.2)	3 (20.0)	0 (0.0)	2 (13.3)	5 (33.3)
Nes PHC, Kheda	40	27 (67.5)	6 (15.0)	1 (2.5)	6 (15.0)	13 (32.5)

Figures in parentheses are precentages.

Table 2. Distribution of chloroquine resistant *P. falciparum* cases among migrant population from different states

States	Sensitive cases	Level of resistance			Total resistant
		RI	RII	RIII	
Gujarat	51 (47.7)	13 (56.5)	2 (100)	7 (38.9)	22 (51.2)
Maharashtra	33 (30.8)	4 (17.4)	0 (0)	9 (50.0)	13 (30.2)
Uttar Pradesh	11 (10.3)	5 (21.7)	0 (0)	0 (0)	5 (11.6)
Andhra Pradesh	2 (1.8)	1 (4.3)	0 (0)	0 (0)	1 (2.3)
Bihar	3 (2.8)	0 (0)	0 (0)	2 (11.1)	2 (4.6)
Orissa	6 (5.6)	0 (0)	0 (0)	0 (0)	0 (0)
Punjab	1 (0.9)	0 (0)	0 (0)	0 (0)	0 (0)
Total	107	23	2	18	43

Figures in parentheses are percentages.

difference in chloroquine resistance in foresaid areas was not statistically significant ($t = 1.7608$; $p > 0.05$). The proportion of RIII resistance ranged between 10.5 and 15.0 per cent in all the three study areas. Studies carried out by Pandya *et al.*[6] revealed existence of 32.7, 36.8 and 7.5 per cent RII + RIII resistant *P. falciparum* in Surat, Bharuch and Kheda districts respectively. The RII + RIII resistant *P. falciparum* were recorded 44 per cent in Surat City, 40 per cent in SSN dam site (Bharuch) and 53.8 per cent in Nes PHC (Kheda) during present study. Further analysis of CRPF cases revealed that most of them were migrants from other parts of Gujarat (51.2 per cent) followed by Maharashtra (30.2 per cent), Uttar Pradesh (11.6 per cent), Bihar (4.6 per cent) and Andhra Pradesh (2.3 per cent). However, maximum resistant cases of RIII level were detected in the migrants from Maharashtra (Table 2).

Response of malaria parasites to chloroquine in our country vary widely. Reduced susceptibility of *P. falciparum* to chloroquine is being reported from different regions and is increasing in extent and degree.[7,8] Results of present investigation confirm the previous reports of *P. falciparum* resistance to chloroquine in southern parts of Gujarat. Existence of varied degree of *P. falciparum* resistant strains among migrants in slums of Surat City, SSN dam site, Bharuch and Nes PHC, Kheda is a matter of serious thought. Aggregation of labourers with high *P. falciparum* infection would provide opportunity to different parasite strains for hybridization. Anti-mosquito measures have diminished in effectiveness in most parts of Gujarat, owing to emergence of resistance in vector species *An. culicifacies* and *An. stephensi* to conventional insecticides[9] and various other factors. Taking advantage of a series of epidemiological factors related to human migration and habit, parasite characteristics, vector patterns and decreasing effectiveness of malaria control measures, CRPF strain may further spread to other areas. To contain *P. falciparum* problem and to liquidate the spread of resistant strains to other areas, the transmission should be curbed by effective insecticidal spray. Detection and treatment services along with health education should be strengthened to avoid prolonged drug pressure resulting from inadequate treatment.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. V.P. Sharma, former Director, Malaria Research Centre, Delhi for his encouragement and providing necessary facilities for the study. We are thankful to Dr. R.S. Yadav, Officer-in-Charge for his valuable suggestions and help. Thanks are also due to Medical Officers of Municipal Corporation Hospital, Surat, Primary Health Centre, Nes and J.P. Hospital, Kevadia, Bharuch for their cooperation in this study. Technical assistance of Mr. S.M. Banerjee and other staff is gratefully acknowledged.

REFERENCES

1. Anon. (1990). The clinical management of acute malaria. *WHO SEARO, Ser.* No. **9:** 75–110.
2. Karmakar, P., S.C. Dutta, M.V.V.L. Narasimham and R.C. Sharma (1990). Status of *Plasmodium falciparum* resistance to chloroquine in Gujarat, Rajasthan and Maharashtra state of India. *Indian J. Malariol.*, **27:** 101–109.
3. Sharma, R.C. and V.P. Sharma (1988). Epidemiological implications of population migration: Pt II. Evidence of chloroquine resistant *P. falciparum* malaria in Kheda district, Gujarat. *Indian J. Malariol.*, **25:** 117–118.
4. Rieckmann, K.H. (1990). Monitoring the response of malaria infections to treatment. *Bull WHO,* **68:** 759–760.
5. Das, Biswaroop (1994). *Socio-economic Study of Slums in Surat City* (Centre for Social Study, Surat): 58–60.
6. Pandya, A.P., R.S. Sharma and G.C. Sahu (1995). An analytical presentation of drug resistance in *Plasmodium falciparum* and guidelines to formulate a drug strategy. *J. Com. Dis.*, **27:** 44–54.
7. Sharma, V.P. (1984). Drug resistant *Plasmodium falciparum* malaria in India. *Proceedings of Indo-UK Workshop on Malaria* (Malaria Research Centre, Delhi): 169–184.
8. Sharma, G.K. (1986). *Malaria and its Control in India*, v. 1. (Directorate of National Malaria Eradication Programme, Delhi): 229–232.
9. Anon. (1991). *Annual Report* (National Malaria Eradication Programme, Delhi): 1–56.

Indian Journal of Malariology
Vol. 37, March–June 2000, pp. 43–45.

Insecticide Susceptibility Status of *Anopheles stephensi* (Liston) in Calcutta, West Bengal

SANGITA CHAKRABORTY[a] and NEELAM TANDON[a]

Keywords: *Anopheles stephensi,* Insecticide susceptibility, Resistance

Use of insecticides is one of the several methods to control larval and adult population of malaria vector(s).[1,2] Of the six primary vectors of malaria in India,[3] *Anopheles stephensi* is the principal transmitter of the disease in Calcutta metropolis,[4] which has been reeling under the grip of malaria since 1995.[5] A notable change in the insecticide susceptibility status of *An. stephensi* has occurred in the metropolis during the last two decades.[6-8] As per recommendations of WHO,[9] the susceptibility status of *An. stephensi* larvae and adults was redetermined after a span of five years.

The tests were performed on larvae collected from the breeding habitats in central, eastern and south-western parts of the metropolis and the emerged females were provided a blood meal prior to the test. A set of control for each experiment was also kept.

The susceptibility of *An. stephensi* larvae to malathion (3.125 mg/l), fenitrothion (0.125 mg/l), fenthion (0.05 mg/l) and temephos (0.25 mg/l) was determined as per WHO guidelines.[9,10]

The average mortality (four replicates) of larvae against each larvicide in each of the above areas was determined , and it was observed that *An. stephensi* larvae were susceptibile to malathion, fenitrothion and fenthion and resistant to temephos (Table 1). The test kit was provided by the Directorate, NAMP, Delhi. The larvicides used in the metropolis are fenthion (Baytex), temephos (Abate) and bacticide respectively.

[a]Department of Medical Entomology, Calcutta School of Tropical Medicine, Calcutta–700 073, India.

Table 1. Susceptibility status of *An. stephensi* larvae to larvicides at diagnostic concentrations in Calcutta metropolis (1998–1999)

Insecticides	Larvicide conc. (mg/l)	Exposure time (h)	No. exposed	Mortality (%)	Susceptibility status
Malathion	3.125	24	100	100	S
Fenitrothion	0.125	24	100	100	S
Fenthion	0.05	24	100	100	S
Temephos	0.25	24	100	88	R
Control	–	24	100	Nil	

The average mortality of larvae against each larvicide in each area was almost same; Water temp. 25.5–27.5°C; RH 78–85 per cent; S — Susceptible; R — Resistant.

Table 2. Susceptibility status of *An. stephensi* adults to adulticides in Calcutta metropolis during 1998–1999

Adulticides	Adulticides conc. (%)	Exposure time (min)	No. exposed	No. of mosquitoes dead 24 h	Mortality (%)	Susceptibility status
Malathion	5	60	100	80	80	R
DDT	4	60	100	80	80	R
Dieldrin	0.2	60	100	12	12	R
Propoxur	0.1	60	100	24	24	R
Fenitrothion	1	120	100	100	100	S
Control	–	60	100	Nil	0	

The average mortality of female (adult) against each adulticide in each area was almost same; Water temp. 24–28°C; RH 68–78 per cent; R — Resistant (mortality < 80 per cent); S — Susceptible (mortality > 98 per cent).

Susceptibility of the blood fed female *An. stephensi* was determined[9] against malathion (5 per cent), DDT (4 per cent), diledrin (0.4 per cent), propoxur (0.1 per cent) and fenitrothion (1 per cent) in separate tests and the average mortality of four replicates in each area was recorded.[10] *An. stephensi* adults were found to be susceptible to fenitrothion and resistant to DDT, dieldrin, malathion and propoxur (Table 2).

DDT (50 per cent) had been sprayed to provide coverage to 50–60 per cent population in West Bengal during 1995–1998 as per infor-

mation from CMOH, Calcutta. Pyrethrum (2 per cent extract — one part in 19 part diesel) and malathion (five litre technical mixed with 95 litre of diesel) is being used for indoor and outdoor fogging respectively, since the last two years, while use of cyfluthrin (@ 25 mg/m^2) for indoor spraying has also been initiated recently.

The susceptibility status of arthropod vectors is influenced by ecology and genetics of the vector population, but is reported to alter by indiscriminate use of insecticides.[11] This explains the difference in insecticide susceptibility status of the same vector species in different geographical areas.[12]

ACKNOWLEDGEMENTS

The authors express their gratitude to the Director, Calcutta School of Tropical Medicine, Calcutta to publish the paper. Thanks are also due to Mr. Manas Kumar Pal for technical and Mr. Sukumar Das for secretarial assistance. Thanks are also due to Directorate NAMP, Govt. of India, Delhi for supplying the WHO test kit.

REFERENCES

1. WHO (1963). Insecticide resistance and vector control. *XIII Report of WHO Expert Committee on Insecticides. Tech. Rep. Ser.* No. **265:** 51–55.
2. Sharma, G.K. (1987). A critical review of the impact of insecticidal spray under NMEP on malaria situation in India. *J. Com. Dis.*, **19**(3): 187–290.
3. Nagpal, B.N. and V.P. Sharma (1994). *Indian Anophelines* (Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi).
4. Siddons, L.B. (1946). *Anopheles stephensi* as a vector of malaria in Calcutta. *J. Mal. Inst. India*, **6:** 367–376.
5. Tandon, Neelam and Sangita Chakraborty (1998). Behavioural factors of *Anopheles stephensi* – The principal vector of malaria in Calcutta City. *Indian J. Pub. Hlth.*, **XLII (2):** 48–49.
6. Hati, A.K., N. Tandon and A.K. Mukhopadhyay (1978). Double resistance in *Anopheles stephensi* in Calcutta. *Indian Med. Gaz.*, CXII(2): 2–24.
7. Mukhopadhyay, A.K., S. Chakraborty, P.K. Karmakar and P. Banerjee (1996). Insecticidal susceptibility status of *Anopheles stephensi* (Liston) in selected areas of Calcutta. *Indian J. Pub. Hlth.*, **XL**(4)**:** 130–133.
8. Roop Kumari, B.R. Thapar, R.K. Dasgupta, S.M. Kaul and Shiv Lal (1998). Susceptibility status of malaria vectors to insecticides in India. *J. Com. Dis.*, **30**(3): 179–185.
9. WHO (1970). Insecticidal resistance and vector control. *Tech. Rep. Ser.* No., 443: 45–79.
10. WHO (1986). Resistance of vectors and reservoirs of disease to pesticides. *X Report of the WHO Expert Committee on Vector Biology and Control. Tech. Rep. Ser.* No., **737:** 49.
11. WHO (2000). Implementation Status of the Global Malaria Control Strategy. XX Report. *Tech. Rep. Ser.* No., **892:** 7–64.
12. Rao, T.R. (1984). *The Anophelines of India.* Rev. edn. (Malaria Research Centre, ICMR, Delhi).

CASE REPORT

Indian Journal of Malariology
Vol. 37, March–June 2000, pp. 46–48.

Cerebellar Ataxia in Falciparum Malaria — A Report of Two Cases

Keywords: Cerebellar ataxia, Falciparum malaria, Trunkal ataxia

Cerebellar ataxia has been described as a complication of variety of infections notably typhoid.[1,2] It may appear as a delayed complication of falciparum malaria after asymptomatic interval of 2–3 weeks.[3] Acute cerebellar syndrome is infrequently encountered as a presenting feature of malaria.[2] Here we are reporting two patients of falciparum malaria who presented with fever and ataxia without loss of consciousness because of its uncommon occurrence.

Case 1

SKR, a 22 year old man from malaria endemic area was admitted with history of high grade fever with chill and rigor for three days and unsteady gait with slurring of speech for one day. The patient was of average built and fair nutrition. He was febrile (Temp. 104°F) and a pulse rate of 130 bpm. He was conscious and cooperative. He had bilateral cerebellar signs as evidenced by finger nose and heel-knee-chin incoordination, intention tremor, dysdiadochokinesia, staggering ataxic gait and scanning cerebellar speech. There was no nystagmus and signs of meningeal irritation. Abdominal examination revealed no hepatosplenomegaly.

Peripheral blood smear was stained with Giemsa which showed asexual forms of *P. falciparum*. The parasite count which was calculated by number of parasites/200 WBC x TLC was 12,250/

mm^3. The CBC, showed TLC – 9800/mm^3, Hb – 8 g per cent, DLC–N, 72 per cent; L, 22 per cent; E, 4 per cent; M, 1 per cent and B, 1 per cent. The biochemical parameters were as follows—FBG, 60 mg per cent; B. urea, 22 mg per cent; S. creatinine, 0.2 mg per cent; S. bilirubin, 0.8 mg per cent; ALT, 22 IU and AST 32 IU. CT scan was within normal limits. CSF analysis showed clear fluid with normal pressure. CSF sugar was 40 mg per cent and protein 65 mg per cent. There were 10 cells/mm^3 and all were lymphocytes.

A diagnosis of falciparum malaria with cerebellar ataxia was made. The patient was treated as severe falciparum malaria with Inj. Quinine dihydrochloride 600 mg. I.V. eight hourly. Patient became afebrile after 72 hours. Parasite clearance and improvement of cerebellar signs occurred 80 hours and 120 hours (five days) after initiation of treatment.

Case 2

RD, a 20 year old man was admitted with history of fever for five days, and shaking of body and unsteadiness of gait for two days. The patient was of average built and anaemic. The temperature was 105°F and pulse rate, 140 bpm. Neurological examination revealed that the patient was conscious and cooperative with cerebellar signs. Those were trunkal ataxia, heel-knee-shin incoordination and the nystagmus which was phasic, horizontal and bidirectional. Deep tendon reflexes were diminished with flexor plantar response.

The haemotological investigations were as follows — Hb, 6.8 g per cent; TLC, 7200; Differential count: N, 70 per cent; L, 20 per cent and E, 10 per cent. Peripheral smear showed ring form of *P. falciparum* with parasitaemia of 7560/mm^3. The biochemical investigations were: FBG, 72 mg per cent; B. urea, 20 mg per cent; S. creatinine, 0.8 mg per cent; S. bilirubin, 0.6 mg per cent; AST, 20 IU/L; ALT, 22 IU/L. CSF analysis was within normal limits. CT scan was normal. The patient was treated with Inj. Quinine dihydrochloride 600 mg per eight hourly intravenously. Fever subsided after 96 hours. Parasitic clearance occurred after 48 hours. The cerebellar signs improved after six days of treatment.

DISCUSSION

Of all neurological manifestations, cerebellar ataxia is a rare complication in falciparum malaria. It can occur as an early, acute cerebellar syndrome with fever[3,4] or as a delayed ataxia after subsidence of fever.[5,6] Stray cases have been reported of the former variety from India and Sri Lanka.[3,4]

The neurological signs in the present cases suggested impairment of cerebellar functions. The early onset of ataxia after fever with a positive blood film and the disappearance of symptoms after antimalarial treatment suggested a causal relation between the malaria attack and ataxia.

The possible mechanism of early cerebellar syndrome in acute malaria is the sequestration of parasitised RBC (PRBC) in the microcirculation resulting in organ dysfunction. In the autopsy study it had been observed that in the cerebellum, the percentage of microvessels with PRBC sequestration was higher than in the cerebrum.[7] Higher degree of vascularity in the cerebellum (seven vessels/mm^2) than in cerebrum (five vessels/mm^2) has been ascribed as the explanation.[7] In spite these, the incidence of cerebellar ataxia is uncommon in malaria.

Clinically and pathogenetically the delayed cerebellar ataxia in falciparum malaria seems to be different from early acute ataxia in many ways. The discrepancy in the time sequence between fever and onset of ataxia in the former, makes a vascular mechanism less likely. Therefore, re-activation of a neurotropic virus, immunological mechanisms or infection by a mutant strain of *P. falciparum* have been suggested.[6]

Though acute cerebellar ataxia has not been included in the classification of severe malaria, we have treated both the cases in that line.[2] On treatment, patients became afebrile after 72 and 48 hours; parasitic clearance after 80 and 48 hours, and disappearance of cerebellar signs after five and six days in cases 1 and 2 respectively.

In conclusion, acute cerebellar ataxia complicating falciparum malaria is essentially a reversible syndrome which takes about a week to subside with the treatment. Hence when cerebellar ataxia develops in the course of febrile illness, patient should also be investigated for malaria infection.

M.K.C.G. Medical College Hospital
Berhampur–760 004, India.

M.K. MOHAPATRA
B.K. PANDA
S.P. DAS

REFERENCES

1. Misra, G.C., S.P. Singh, M.K. Mohapatra, P.K. Prusty and R.K. Das (1985). Typhoid cerebellitis. *J. Indian. Med. Assoc.*, **83**(10): 352–353.
2. Warrell, D.A. (1993). Clinical features of malaria. *Bruce-Chwatt's Essential Malariology*. Eds. H. M. Gilles and D.A. Warrell. III edn. (Edward Arnold, London): 35–48.
3. Chaddha, V.S. and R.P. Kothari (1978). Cerebellar syndromes in malaria. *J. Assoc. Phy. India*, **26:** 445–446.
4. Illagasekera, V.L.U. and H.J. deSilva (1976). Acute cerebellar syndrome in falciparum malaria. *Ceylon Med. J.*, **22:** 130–132.
5. Kumawat, B.L., D.K. Kochar and S.K. Kochar (1999). Delayed cerebellar ataxia – A complication of falciparum malaria (abstract). *J. Assoc. Phy. India.*, **47:** 143–144.
6. Senanayake, N. (1987).Delayed cerebellar ataxia : A new complication of falciparum malaria. *British Med. J.*, **294:** 1253–1254.
7. Sein, K.K., Y. Mueno, H.V. Thue, T.K. Ann and M. Aikawa (1993). Differential sequestration of parasitized erythrocytes in the cerebrum and cerebellum in human cerebral malaria. *American J. Trop. Med. Hyg.*, **48**(4): 501–511.

STATEMENT ABOUT OWNERSHIP AND OTHER PARTICULARS OF THE *INDIAN JOURNAL OF MALARIOLOGY*

FORM IV

(See Rule 8)

1.	Place of publication	Malaria Research Centre (ICMR) 22 Sham Nath Marg Delhi-110 054
2.	Periodicity of its publication	Quarterly
3.	Publisher's Name Nationality Address	Dr. Sarala K. Subbarao Indian Malaria Research Centre (ICMR) 22 Sham Nath Marg Delhi–110 054
4.	Printer's Name Nationality Address	Dr. Sarala K. Subbarao Indian Malaria Research Centre (ICMR) 22 Sham Nath Marg Delhi–110 054
5.	Editor's Name Nationality Address	Dr. Sarala K. Subbarao Indian Malaria Research Centre (ICMR) 22 Sham Nath Marg Delhi–110 054
6.	Name and addresses of individual who own the newspaper and partners or shareholders holding more than one per cent of the total capital	Malaria Research Centre (ICMR) 22 Sham Nath Marg Delhi–110 054

I, Sarala K. Subbarao, hereby declare that the particulars given above are true to best of my knowledge and belief.

Sd/-
(SARALA K. SUBBARAO)
(Signature of Publisher)

16 April 2000