PRINT: ISSN 0970-9274 ONLINE: 2456-6608
DOI: 10.31901/24566608.1993/04.02.07
JOURNAL OF HUMAN ECOLOGY
J. Hum. Ecol., 4(2): 125-129 (1993)

Seroepidemiology of Malaria in Bastar District, Central India

Archana Thakur, Ishwar C. Verma and P. S.N. Menon
Genetic Unit, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi 110 029, India

KEY WORDS Enzyme Linked Immunosorbent Assay. Malarial Antibody. Seropositivity.

ABSTRACT We assayed malaria antibodies by an enzyme linked immunosorbent assay employing A-18 soluble antigen from FCB-2 strain of *P. falciparum* in 472 Muria Gond tribals (age range 5 to 65 years) in Bastar district, Madhya Paradesh, and 100 subjects in Delhi. In Muria Gonds, the seropositivity rate for malaria antibodies increased upto 30 years of age. It plateaued at age group 31-40 years and beyond. At each age group the seropositivity was significantly greater in Muria Gonds as compared with that in Delhi subjects ($p = 0.0004$).

INTRODUCTION

Parasite index and spleen rate have been the traditional indices for determining endemicity of malaria (Voller and Draper, 1982). The former gives information about point prevalence of malaria, but has limitations in populations which have achieved some degree of immunity, and have experienced frequent use of antimalarials for thereapeutic and prophylactic purposes (Voller and Bruce-Chwatt, 1968). The spleen rate, although a rapid method for estimating endemicity is only a rough guide, and is most useful where malaria is stable. In contrast, serological tests for malarial antibodies (Jefferg et al., 1975; McWilson et al., 1975a, b and WHO, 1975) provide information about period prevalence of malaria, and reflect the total immunologic experience of the individual or the collective group for infection with malarial parasite. The spectrum of humoral antibody response in malaria is determined by age, immunologic competence, the degree of exposure to malaria antigens and the intensity of therapy (McGregor et al., 1965 and McGregor, 1974).

Amongst the various serological tests for malaria for monitoring period prevalence, (Ray et al., 1983; Bhat and Srinivasa, 1983; Mahajan and Ganguly, 1984; and Kumar et al., 1986) measurement of antibody levels by enzyme linked immunosorbent assay (ELISA) is particularly useful for epidemiological application, as it requires single dilution and only small quantity of the blood sample, which can be collected on filter paper. We have used ELISA for detecting antibodies against malarial parasites in subjects from Bastar district, Central India, an area known to be endemic for malaria, as well as in Delhi, which has low prevalence of malaria.

Reprint Requests : Professor I.C. Verma

MATERIAL AND METHODS

Study area

The present study was conducted in Geedum and Pharasgaon blocks of district Bastar in Central India. These are known to be endemic for malaria (Annual parasite index for Geedum and Pharasgaon blocks was 24.6 and 18.45 respectively, in 1987). A multistage stratified random sampling procedure was used throughout the study (first stage-selection of Bastar district which has a tribal population of more than 60% ; second stage-selection of blocks, one from north and one from south Bastar; third stage was the selection of villages

on the basis of the distance from the health centre. We chose 100 house-holds in each block, and about 500 individuals belonging to Muria Gond tribe were identified for the study. The data obtained was compared with persons randomly selected from Delhi.

Sample Collection

All the identified individuals were requested to give blood. From those who agreed, blood samples were collected on filter paper (Whatman No.3), dried and stored at 4° C. At the time of assay serum was eluted from the filter paper strips by soaking 7 mm discs in 0.3 ml phosphate buffer, pH 7.4, for 2 hours. Concentration of the elute was 1:16 which was further diluted 1:256 by phosphate buffered saline.

ELISA Procedure

The malarial antibodies were assayed by ELISA technique of Spencer (Spencer et al., 1979) with minor modifications (Tomar, 1986). The A-18 soluble antigen derived from *Plasmodium falciparum* strain FCB-2 was used to coat the microtitre plates (Immunol-2 Dynatech, Denmark) to an optimal concentration. This antigen crossreacts with both *P. falciparum* and *P. vivax*. ELISA was standardized for optimal incubation time (90 minutes), conjugate dilution (1:4000), sera dilution (1:256) and elution of sera from dried blood on filter paper (Tomar, 1986). The antimalarial antibodies in human serum were quantitated at a single dilution using horse radish peroxidase (HRPO) labelled with antihuman IgG followed by the chromogenic enzyme substrate orthophenylene diamine. The readings were taken in an ELISA reader at 492 nm. Samples giving optical density more than 0.25 at 1:16 dilution were considered positive. Positive reference sera H-350, negative reference sera as well as the malarial antigen (A-18) were kindly supplied by Dr. C. Espinal, Columbia, South America.

The ELISA technique was validated by comparing the seropositivity in 100 cases with indirect immuno-fluorescent (IIF) assay as per the method of Sulzer et al. (Sulzer et al., 1969), and indirect heamgglutination assay (IHA) according to Meuwissen with minor modifications (Meuwissen, 1979). IIF, IHA and ELISA were positive in 83, 79, and 81 per cent of cases, respectively; thus, were equally good in detecting antibodies against malaria (P=0.77). Comparison of seropositivity was done by using Student's *t*-test with the computer program Epistat, a Statistical package designed by TL Gustafson, 1705 Gattis Road, Round Rock, Texas-78664 (1984).

RESULTS

Sensitivity and Specificity of ELISA

Sensitivity of ELISA was tested by assaying sera from 25 patients with acute *P. falciparum* malaria and 25 healthy individuals from Jammu and Kashmir who were never exposed to malaria in the past. Patients' sera demonstrated optical density >.25 in dilutions ranging from 1:16 — 1:256 whereas in sera from non-malarial subjects optical density was <.25 even in <1:16 dilution. Therefore postive titres were considered to be >.25. Soluble antigent A-18 derived from *P. falciparum* strain FCB -2 was cross reacting with *P. vivax* antigen also.

Stability of the Stored Samples

Blood samples collected on filter paper strips, dried and stored at room temperature for at least 2 weeks and at 4° C for 1 year tested without any loss of antibody titres.

The age distribution of 472 Muria Gonds and 100 subjects from Delhi is shown in table 1. The seropositivity for malarial antibodies and geometric mean titre in different age groups is also displayed.

Among the Muria Gonds, the seropositivity increased from the age group 5-10 years to 31-40 years. It plateaued around 88-90 % at

Table 1: The percentage seropositivity, mean optical density (OD), geometric mean titre (GMT) for malaria antibodies among Muria Gonds (MG) and Delhi subjects (D) in different age groups

Age (yr)		*Total No.*	*Sero -ve OD < .25*	*Sero +ve OD < .25*	*% Sero +ve*	*O.D. (Mean ± SD)*	*GMT*
5-10	MG	76	24	52	68.4	0.54 ± 0.30	0.3
	D	12	8	4	33.3	0.32 ± 0.16	0.3
11-20	MG	104	25	79	76.0	0.68 ± 0.31	0.4
	D	20	17	3	15.8	0.36 ± 0.18	0.3
21-30	MG	131	25	106	80.9	0.60 ± 0.26	0.4
	D	30	17	13	43.3	0.48 ± 0.22	0.3
31-40	MG	83	10	73	88.0	0.62 ± 0.24	0.4
	D	17	7	11	64.7	0.50 ± 0.16	0.4
41-50	MG	47	5	42	89.4	0.70 ± 0.28	0.4
	D	11	8	3	28.6	0.52	0.4
51	MG	31	3	28	90.5	0.78 ± 0.39	0.4
	D	10	7	3	.0	0.26 ± 0.11	0.2
Total	MG	472	92	380	80.5		
	D	100	63	37	36.7		

the age group 31-40 years and beyond. Statistical comparisons between age group 5-10 years (68.4% seropositivity) differed significantly only from the age group 31- 40 years and above (P=0.0004). The seropositivity at age 11-20 years did not differ from age group 21-30 years (P=0.45), although it differed significantly when compared with age groups 31-40 years and above (P=0.0057).

In *subjects from Delhi* age groups of 5-10 and 11-20 years as well as 41-50, 51-60 and 60+ years were combined as the number in different age groups was small. Statistical analyses for seropositivity for the age groups 5-20 years, 21-30 years, 31-40 years and beyond did not show significant differences (P=0.0796).

The seropositivity among Muria Gonds was compared with that in subjects from Delhi. The percentage of seropositivity between Muria Gonds and Delhi subjects in the various age groups differed significantly (P=0.0040). Among subjects from Delhi only 36.7% of the individuals showed malarial antibody titre more than 0.25, while in Muria Gonds 80.5% of the individuals possessed similar antibody levels.

In Muria Gonds, geometric mean titre of antibodies against malaria increased with age (Table 1). However, in Delhi subjects the geometric mean titre did not show any clear cut relationship with age. Malarial antibodies titre (mean optical density) in slide positive cases (n=70) was 0.66, and in slide negative cases (n=402) was 0.50. This difference was not statistically significant.

DISCUSSION

The present study showed a significant age dependent increase of antibodies to malaria among Muria Gonds in Bastar district (P=0.00068). This increase with age is similar to the experience of Bhat and colleagues (Gupta et al., 1983 and Bhat and Srinivas, 1984) in Karnataka, and McGregor et al. in Africa (McGregor, 1974). It reflects greater exposure to malarial parasites (*i.e.* more infections) with advancing age. The plateau of antibody titres was reached in adults in the age group 31-40 years and beyond.

In contrast, in Delhi subjects seropositivity did not show any consistent increase with age, and the values did not differ significantly at

different age groups (P=0.0796). This may partly be because of small numbers studied in the older age groups in Delhi.

There was a striking difference in the seropositivity at different ages in Muria Gonds as compared with Delhi subjects (P=0.00068). This reflects the presence of greater infection with malarial parasites in Bastar district, as compared with the population in Delhi.

ELISA is comparatively rapid, simple and inexpensive for large scale seroepidemiological examinations and also reflects present and past malarial infection. Optical density >1.0 reflect present parasitemia or recent past infection while >.25 O.D reflect low antibody titre (1:16), which shows that individual was exposed to malarial infection 6-7 months before.

Regarding methodology of ELISA, the critical component is the antigen used. We employed A-18 soluble antigen derived from *P. falciparum* strain FCB-2. However, other Indian workers have used *P. knowlesi* (Dutta et al., 1982 and Tandon et al., 1982) and *P. falciparum* (Mahajan et al., 1981 and Bhat and Srinivas, 1984). The results of studies done in different countries all over the world (Spencer et al., 1979) including India (Ray et al., 1981; Francis et al., 1982; Walters and McCulculchan, 1982; Gupta et al., 1984), have shown that the *in vitro* continuous culture of asexual stages of *P. falciparum* provide an unlimited and stable supply of plasmodium antigen for serology of vivax and falciparum malaria. However, the advent of recombinant DNA technology has led to the development of very precise and accurate diagnostic tests for the detection of different species of malarial parasite. These probes will be invaluable tools for epidemiologic investigation of malaria, and for monitoring the malaria control and eradication programmes (Walters and McCulchan, 1982).

ACKNOWLEDGEMENT

We thank the Indian Council of Medical Research for financial assistance for carrying out this study.

REFERENCES

Bhat, P. and Srinivasa, H.: Seroepidemiology of malaria in India. In: *The Proceeding of the Indo-UK Workshop on Malaria*, New Delhi. Malaria Research Centre Publication. P 239-43 (1984).

Datta, G.P., Srivastava, I.K., Sharma, P., Nath, A., Agarwal, S.S. and Dwivedi, S.R. : Enzyme linked immunosorbent assay test in diagnosis of human malaria. *Indian J. Malariology, 19*: 33-37 (1982).

Francis, V.S., Gupta, M.M. and Bhat, P. : Evalution of crude soluble antigen from *in vitro.* culture of *P. falciparum* for ELISA. *Trop. Med. Parasitol., 33 :* 240-244 (1982).

Gupta, M.M., Sebastian, M.J., Bhat, P. and Label, H.O. : Evaluation of *in vitro* cultured *P. falciparum* for malaria serology. *J. Trop. Med. Hyg., 84 :* 165-170 (1981).

Gupta, M.M., Srinivasa, H. and Bhat, P. : Seroepidemiology of malaria in Karnataka state, Longitudinal study of a population from an area with high incidence at Kolar, South India. *J. Inf. Dis., 148:* 609 (1983).

Jeffery, G.M., McWilson, W., Collins, W.E. and Label, H. : Application of indirect fluorescent antibody method in a study of malaria endemicity in Mato, Grasso, Brazil. *Am. J. Trop. Med., 24 :* 402-411 (1975).

Kumar, R., Rao, K.R., Ansari, M.A., Razdan, R.K., Shrivastava, A. and Sharma, V.P. : Feasibility of 1HA and ELISA in epidemiology of malaria. *Indian J. Malariology, 23:* 75-80 (1986).

Mahajan, R.C., Ganguly, N.K., Thadani, M., Gill, N. and Chandnani, R.E. : Comparison of ELISA with hemagglutination and indirect fluorescent antibody test in malaria. *Indian J. Med. Res., 73:* 874-879 (1981).

Mahajan, R.C. and Ganguly, N.K. : Role of serology of malaria under Indian conditions. In: *The Proceedings of Indo-UK Workshop on Malaria.*, New Delhi. Malaria Research Centre Publication p 233 (1984).

McGregor, I.A., Wlilliams, K., Voller, A., and Billewicz, W.Z. : Immunofluorescence and the measurement of immune response to hyperendemic malaria. *Trans. Roy. Soc. Trop. Med. Hyg., 59:* 395-414 (1965).

McGregor, I.A. : Mechanisms of acquired immunity and epidemiological patterns of antibody responses in malaria in man. *Bull. WHO, 50 :* 259-266 (1974).

McWilson, W., Collins, W.E., Skinner, J.C. and Lerin, A.J. : Seroepidemiology of malaria in middle America. I. Longitudinal studies on populations in a low incidence area of El Salvador. *Am. J. Trop. Med. Hyg., 24 :* 740-748 (1975a).

McWilson, W., Collins, W.E., Jeffery, G.M. et al. : The seroepidemiology of malaria in middle America. II Studies on the Pacific coast of Costa Rica. *Am. J. Trop. Med. Hyg., 24 :* 749 (1975b).

Meuwissen, JHE. The indirect haemagglutination test for malaria and its application to epidemiological sarveillance. *Bull. WHO, 50:* 277-286 (1974).

Ray, K., Rai Chowduri, A.N., Sharma, R.S., Rai, A., Choudhury, D.S. and Label, H.O. : Indirect hemagglutination malarial antibodies in fever cases from a rural community in Alwar district, Rajasthan. *Indian J. Med. Res., 73 :* 78-81 (1981).

Ray, K., Sharma, M.C., Sivaraman, C.A. and Rai Chowdhuri, A.N. : Evaluation of IHA test using *Plasmodium falciparum* from *in vitro* culture and its comparision with IIF and ELISA. *Indian J. Med. Res., 78:* 624-630 (1983).

Spencer, H.C., Collins, W.E., Chin, W. and Skinner, J.C. : The enzyme linked immunosorbent assay (ELISA) for malaria: The use of *in vitro* cultured *Plasmodium falciparum* antigen. *Am. J. Trop. Med. Hyg., 28:* 927-932 (1979).

Spencer, H.C., Collins, W.E., Chin, W. and Skinner, J. : The enzyme-linked immunosorbent assay (ELISA)for malaria. II. Comparison with the malaria indirect fluorescent antibody test (IFA). *Am. J. Trop. Med. Hyg., 28:* 933-936 (1979).

Sulzer, A.J., Wilson, M. and Hall, E.C. : Indirect fluorescent antibody tests for parasitic diseases. V. An evaluation of a thick smear antigen in test for malaria antibodies. *Am. J. Trop. Med. Hyg., 18:* 199-205 (1969).

Tandon, A., Saxena, R.P., Bhatia, B. and Saxena, K.C.: The enzyme linked immunosorbent assay in the immunodiagnosis of human malaria. *Trans. Roy. Soc. Trop. Med. Hyg., 76:* 371-372 (1982).

Tomar, A. : *Interaction of Malarial Infection with Genetic Markers.* Ph.D Thesis, All India Institute of Medical Sciences, New Delhi (1986).

Voller, A. and Bruce-Chwatt, L.J. : Serological malaria surveys in Nigeria. *Bull. WHO, 39 :* 883-897 (1968).

Voller, A. and Draper, C.C. : Immunodiagnosis and seroepidemiology of malaria. *Br. Med. Bull., 38 :* 173-177 (1982).

WHO. : Developments in malaria immunology, *Technical Report Series, 579 :* 29-36 (1975).

Walters, A.P. : McCulculchan, T.F.: Rapid sensitive diagnosis of malaria based on ribosomal RNA. *Lancet, 1:* 1343-345 (1982).