SERO-EPIDEMIOLOGICAL SURVEY OF MALARIA BY IMMUNUOFLUORESCENCE IN PAKISTAN (DISTRICT GUJRANWAIA - PUNJAB)

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ABSTRACT

Indirect Immunofluorescence Antibody (IFA) Technique was employed, for field detection of malarial parasite.The technique involved application of homologous antigen slides of Plasmodium falciparum and P. vivax prepared in the field conditions. Serological study, simultaneously using P. talciparum and P. vivax antigens, brought out a clearer picture of distribution and role of the parasite species in the epidemiology of the disease in the area studied. The survey supported the contention of persistence of transmission of malaria in Gujranwala District (JPMA 41:216,1991).

INTRODUCTION

Immunodiagnostic methods for epidemiological investigatioin and surveillance have gained importance. Of the various immunodiagnostic techniques IndireCt Immunofluorescence antibody (IFA) technique has been extensively employed in malaria studies as it offers the highest degree of sensitivity, specificity and reproducibility[1]. The technique had been applied for sero- epidemiological surveillance of disappearing malaria in Greece, Tunisia, Brazil and middle America[2-5]. Serological surveys may be of value as sensitive indicators of the return of malaria into area where eradication has been previously achieved[7]. Sero- epidemiological survey of malaria by immunofluorescence was done to show that the technique may be introduced in a simple way for field application in malaria control programme to supplement the direct microscopy for epidemiological evaluation. Besides heterologous antigen slides of plasmodium P. fieldi field collected homologous antigen slides of P. falciparum and P. vivax have been advantageously employed, as an improvement of the previous technique[8].

MATERIAL AND METHODS

In September, 1981 three villages - Bupra Kalan, GarmulaVerkan and Uchey Mangat (sectors C-4, D-8 and E-13 of Malaria Control Programme) were selected for the parasitic serologic surveys. The subjects available for the study were school children and adult males. Background information with regards to insecticide sprayings and ACD slide examination records through the years 1978-1981 were obtained from the district health authorities. The population of the villages ranged from 4 to 5 thousands.

In October, 1982 surveys were undertaken in villages Pipli Goraya and Uchey Mangat (MCP sectors D-5 and E-13) and in November 1982 in a small village of Nobarah sharif (MCP sector F-5). The population under consideration in the sectors D-5 and F-5 were within 1-2 thousands.

A single thick and thin blood film (on the same slide) was used for microscopic diagnosis and filter paper sample of 0. 1mm of blood obtained from the same finger puncture in the manner already described in detail earlier8.

In IFA serological study, antigen slides of P. fieldi were employed for sera samples of sector C-4 and D-8. Antigen slides of P. falciparum were used for sera samples, collected in September, 1981 from sector E-13.

Since the study was being switched over from heterologous antigen of P. fieldi to homologous antigen of P. falciparum it was considered necessary to compare the sensitivity of the antigen slides of P. fieldi prepared from heavily infected monkey's blood in the laboratory and P. falciparum antigen slides of heavily infected blood of a patient in the field.

Sera samples collected in October/November, 1982 from sectors D- 5, E-13 and F-5 were tested against P. falciparum and P. vivax antigens simultaneously, with slides prepared from patients' blood in the field.

Antigen slides of P. fieldi, P. falciparum and P. vivax were prepared locally from heavily infected blood samples following the method of Sulzer et al[9].

I. F.A. test procedures were in accordance with the memorandum of serological testing of malaria[10] and serodiagnosis of malaria[11] as reported earlier in detail[8].

RESULTS

Information on the epidemiological situation in the study area, sectors C-4,D-8 and E-13 is summarized in Table I.

TABLE I. Parasite positivity of a CD Slide examination in three Sectors.

	Sector D-8 Garomula Varken				Sector C-4, Bupra Kalan					Sector-E-13 Uchey Manget				
N0. Ex.	No. Positive	P.V.	Pf.	Spray	No. Ex.	No. Posit.	P.V.	Pf.	Spray	No. Ex.	No. Positive	P.V.	Pf.	Spray
545	0	0	0	Yes	580	0	0	0	Yes	430	0(0%)	0	0	Yes
458	0	0	0	Yes	402	2(0.5%)	2	0	No	416	2(0.48%)	2	0	No
558	33(5.9%)	32	1	Yes	417	21(5.0%)	21	0	Yes	412	14(3.4%)	14	0	Yes
581	30(5.19%)	30	0	Yes	600	1(0.16%)	1	0	Yes	450	5(1.1%)	5	0	Yes
103	7(6.8%)	7	0		142	4(2.8%)	3	1		95	21(22.1%)	2	19	

According to active Case Detection (ACD) slide examination results, in 1978, all the three sectors remained free from the disease. Two cases in each of the sectors C-4 (0.5%) and E-13 (0.48%) were recorded in 1979 and by the year 1980 malaria seems to have reappeared in all the three sectors, inspite of annual insecticide spraying operations.

ACD records of 1981 revealed decline in cases. In the surveys conducted by N.I.H. in 1981 in the three sectors parasite rates higher than ACD findings were recorded. Parasite rates of 6.8% and 2.8% were recorded in Sectors C-4 and D-8 respectively, conforming to the higher and lower rates in the two sectors. The parasitological picture of Sector E-13 was entirely different from ACD records ofMCP. A parasite rate of as high as 22.1% was recorded. Another significant feature was species distribution, 90.5% being Plasmodium falciparum infections, contrary to the previous records of only P. vivax in all ACD findings.

The parasitologic survey results of sectors D-5, E-13 and F-5 are shown in Table II.

TABLE II. Parasitologic survey of malaria M.C.P. Sector Gujranwala.

Sector	No.ex.	Posit.	%	SPECIES			
				Pv.	%	Pf.	%
D-5	48	8	16.7	7	87.5	2	25
E-13	76	30	39.47	4	13.3	28	93.3
E-5	11	5	45.5	0	-	5	45.5

It may be observed that in respect of these sectors previous records of ACD slide examination carried out by MCP were not considered necessary. I.F.A. serologic survey results are summarized in Table III.

TABLE III. I.F.A. Serology of blood samples of M.C.P. Sectors of Gujranwala district.

Sector	Age Group	No. Ex.	No. Posit	I.F.A. Titres								
				20	40	80	160	320	640	1280	Mean	GMRT
D-8	5-10 Yrs	48	36 (75%)	6	14	12	3	1	-	-	1: 68	1.73
	11-20 Yrs	38	30 (78.9%)	6	10	9	4	1	-	-	1: 73	1.74
	20 Yrs	17	13 (76.47%)	0	4	3	5	1	-	-	1:117	1.97
	TOTAL	103	79 (76.7%)	12	28	24	12	3	-	-	1: 78	1.77
C-4	5-10 Yrs	51	41 (80.39%)	12	14	12	3	-	-	-	1:55	1.65
	11-20 Yrs.	48	29 (60.41%)	13	10	4	2	-	-	-	1:45	1.55
	20 Yrs.	43	26 (60.5%)	7	10	8	1	-	-	-	1:52	1.63
	TOTAL	142	96 (67.65%)	32	34	24	6	-	-	-	1:51	1.61
E-13	5-10 Yrs.	15	10 (67.6%)	3	5	1	1	-	-	-	1: 50	1.60
	11-20 Yrs.	34	26 (67.5%)	6	5	3	6	3	3	-	1:169	1.95
	20 Yrs.	46	41 (89.1%)	6	3	9	8	7	3	5	1:312	2.17
	TOTAL:	95	77 (81.0%)	15	13	13	15	10	6	5	1:230	2.02

GMRT: antilog Ef(log x) N where x is the reciprocal titre N is the number sera with positive IFA and f is the number of sera with a given titre.

Serologic survey results of sectors D-5, E-13 and F-S are summarized in Table IV.

TABLE IV. Serologic Survey of malaria (I.F.A.) M.C.P. Sectors Gujranwala, October, 1982.

Sector	Ag.	Ex.	(P)	%	I.F.A. TITRES 1:20	1:40	1:80	1:160	1:320	1:640	MEAN
D-5	Pf/Pv	47	(35)	72.9							
	Pf.	47	(10)	21.3	4	4	-	2	-	-	1:56
	Pv.	47	(34)	72.3	7	10	10	5	1	1	1:92
E-13	Pf/Pv	65	(49)	75.4							
	Pf.	65	(27)	41.5	2	5	10	5	4	1	1:139
	Pv.	65	(45)	69.2	8	13	20	5	-	-	1:67
F-5	Pf/Pv	11	(10)	90.9							
	Pf.	11	(4)	36.4	-	-	2	1	1	-	1:140
	Pv.	11	(10)	90.9	4	4	2	-	-	-	1:48

These serologic surveys were conducted using P. falciparum and P. vivax antigens to provide a broader and in depth- picture of the epidemiological situation of the disease in the area with reference to specific load of infection.

A comparative broader picture of parasitologic, serologic data of the surveys undertaken in the various sectors is presented in Table V.

TABLE V. Parasitologic Serologic surveys of malaria M.C.P. Sectors, Gujranwala Sept. 81/Oct. 82.

sectors	Parasitologic Surveys					Serologic surveys					
	Ex.	(P)	%	Pf.	Pv	Ag.	Ex.	(P)	Seroposit %	Mean titre	GMRT
C-4	142	(4)	2%	1	3	Pfd	142	(96)	67.68%	1:51	1.61
C-8	103	(7)	6.5%	0	7	Pfd	103	(79)	76.7%	1:78	1:68
E-13 (Sept. 1981)	95	(21)	22.1%	19	2	Pf	95	(77)	81.0%	1:230	2.02
D-5	47	(8)	16.7%	2	7	Pf/Pv	47	(35)	72.91%		
						Pf	47	(10)	21.3%	1:56	1.16
						Pv	47	(34)	72.3%	1:92	1.78
E13	76	(30)	39.5%	28	4	Pf/Pv	65	(49)	75.4%		
						Pf	65	(27)	41.5%	1:139	1.98
						Pv	65	(45)	69.2%	1:67	1.75
F-5	11	(5)	45.5%	5	0	Pf/Pv	11	(10)	90.9%		
						Pf	11	(4)	40.0%	1:140	1.98
						Pv	11	(10)	90.9%	1:48	1.57
(Oct., 1982)											

DISCUSSION

ACD slide examination of results obtained from CP differed with present survey findings. The latter survey was conducted in post-transmission period, when most of the infections of early transmissions were probably the process of regression and dying out, whereas "ACD" records were of the full year including active transmission period, therefore ACD findings should have showed much a higher rates.

The parasite rates of 6.8% and 2.8% in sectors C-4 and D-8, respectively, are higher than the ACD records. The findings conform to the comparative degree of higher and lower rates in the two sectors. The record of parasite rate of 22.1% in sector E-13 was Out of proportion to the ACD findings and still more surprising is the species prevallence, 90.5% being P. falciparum. It may be observed that ACD examination results did not represent the true epidemiological picture of the disease situation.

Sera samples of sectors D-8 and C-4 were tested against P. fleldi antigen, and those of sector E-13 (of September, 1981) have been tested against P. falciparum antigen. Results of 5 sera samples tested simultaneously against P. fieldi and P. falciparum were similar.

It has been shown that of the simian malaria strain P. fieldi can be a good substitute for P. falciparum as antigen[8,12]. Under the circumstances the serological results of the three sectors, as a whole may be compared.

The seropositivity rates of 67.6%, 76.9% and 81.0% in sectors C- 4, D-8 and E-13, respectively, were to some extent in accordance with similar higher trend of parasite rates of 2.8%, 6.8% and 22.1% respectively in the three sectors. It was further supported by the mean IFA titres plus GMRT ratings in the three sectors.

The parasitological sth-vcys conducted in sector D-5, E-13 and F-5 during October/November, 1982 recorded parasite rates of 16.7%, 39.5% and 45.5%, respectively. The observations of a parasite rate of 45.5% in sector F-S was based upon an examination of only 11 cases, a very small sample and probably only those subjects came for blood examinations who had the clinical experience. Similarly the blood slide examination results of sector E-13 showed a higher parasite rate of 39.5% as compared to previous years result of 22.1%.

Serological study simultaneously using P. falciparum and P. vivax antigens brought out a much clear picture of distribution and role of the parasite species in the epidemiology of the disease in the area. The results of parasitological survey in sector D-5 revealed a predominance of P. vivax, I.F.A. serology showing higher seropositivity (72.3%), higher mean titre (1:92) and GMRT (1:78) with P. vivax antigens uniformly supported the predominance of the species. Lower seropositivity (21.3%), lower mean titre (1:56) and GMRT (1.16) recorded with P. falciparum antigen were in accordance with the lower rate of the species prevalence.

In sector E-13 parasitological survey indicated predominance of P. falciparum at least during the period when the survey was taken. Similar situation was recorded in the previous year. I.F.A. serology presented a different picture and with P. vivax antigen higher seropositivity (69.2%), lower mean titre (1:67) and GMRT (1.75) were recorded whereas with P. falciparum antigens lower seropositivity (41.5%), higher mean titre (1:139) and GMRT (1.75) were observed. In the survey conducted in the sector in the previous year it had lower parasitological rates (22.1%), higher seropositivity (81.0%) higher mean titre 1:204 and GMRT (2.00). This variation may be due to differences in sampling, which may have been uneven.

Similar situation was observed in sector F-5 in a small sample i.e., with P. vivax antigen higher seropositivity, lower mean titre and GMRT were recorded and with P. falciparum antigen, lower seropositivity, higher mean titre and GMRT were recorded.

It may be observed that with P. vivax, higher seropositivity rates were observed in all the three sectors (viz. D-5, E-13, F- 5) irrespective of the degree of species prevalence in the parasitological survey. However, the higher or lower mean titre and GMRT corresponded to the species prevalence. The higher seropositivity with P. vivax antigen on the face of lower species prevalence may be explained by inclusion of some false positives in lower titre level, which seems unlikely. Persistence of residual antibody which persists much longer in P. vivax infections and the cross reaction factor which is always present amongst the species of plasmodia must also be borne in mind 12.13 Parasitic serologic surveys support the contention of persistence of transmission of malaria in the area. However for a better elucidation of the epidemiological situation of the disease, it is observed that the child population below 5 years of age including infants, should have been included in the samples, which was not

achieved due to some obvious reasons.

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