

COMPARISON OF IMMUNOFLUORESCENCE AND ELISA IN THE DETECTION OF MALARIAL ANTIBODIES IN SOUTHERN IRAN

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ABSTRACT

In order to compare the enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody technique (IFAT) in detection of malarial antibodies, 652 sera samples collected in the Health Survey Project (1975) in a random sampling method from about 10% of residents of selected villages in Bandar Abbas and Minab areas of Southern Iran, were tested with the above two serological methods.

In microscopical examination of blood films malaria parasite (*P. vivax*) was found in 12 cases.

Malarial antibodies were detected with Aotus *P. falciparum* and *P. vivax* malaria antigens in titres $1/40$ in 24.3% and 34.9% respectively. The ELISA values with Aotus, *P. falciparum* antigen in 15.6% were more than 0.2. In general, the IFAT showed a considerably higher positivity rate than the ELISA. The results of both types of serological assay indicated a progressive increasing of positivity rate and antibody level with the age.

In the present study malaria antibody was not detected by ELISA in some *P. vivax* parasitologically proved cases; perhaps due to the using

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of heterologous *P. falciparum* antigen. The use of mixed polyvalent *P. falciparum* and *P. fieldi* malaria antigens was more efficient in detecting higher titres of malaria antibodies.

INTRODUCTION

This study presents an extension of the previous work on malaria serology in Iran⁽¹⁾. In earlier studies, indirect immunofluorescence method was used to measure antibody rates and levels to malaria to determine whether this information could be useful for the assessment of the epidemiological studies of malaria in Iran. In 1974 another serological test has been devised for malaria, the microplate enzyme-linked immunosorbent assay (ELISA)⁽²⁾. This communication deals with its application in Iran and a comparison of the results so obtained with those determined with immunofluorescence.

MATERIALS AND METHODS

The sera were prepared in random sampling, by venipuncture of about 10% of residents of selected villages in Bandar Abbas and Minab areas of Southern Iran, in the "Health Survey Project" carried out by the School of Public Health and Institute of Public Health Research, University of Teheran, in 1975.

The area is located on the Northern coast of the Persian Gulf and Oman Sea, 55 - 57° east and 27 - 28° north in a sub-tropical climate.

Malaria was hyperendemic in this area up to 1950. Afterwards the malaria control and eradication programs have considerably reduced the malaria incidence but interruption of transmission was not achieved and it occurs in most months of the year.

P. vivax and *P. falciparum* are prevalent species of plasmodia in this area.

Antimalarial drugs are commonly distributed for prophylaxis and treatment in irregular mass administration.

There was a considerable increase of malaria incidence in these areas in 1974-1975.

From all subjects routine thick blood films were examined microscopically for malaria parasites in Bandar Abbas Field Research Station. In this study, altogether, 652 sera samples were collected in duplicate capillary tubes, they were kept frozen (-20° to -70°C), until examined. One tube was used in the Protozoology Unit, School of Public Health, University of Teheran for tests by IFA technique⁽³⁾ with Aotus

P. vivax and *P. falciparum* malaria antigens (received from N.I.C.M., the Zoological Society of London through W.H.O. grants). The second capillary of sera was used in the laboratory of Infectious Diseases, Nuffield Institute of Comparative Medicine, the Zoological Society of London for microplate ELISA tests⁽²⁾ using sonicated Aotus *P. falciparum* malaria antigen in dilution 1/400 and mixture of equal volume of *P. falciparum* and *P. fieldi* antigens, each with above mentioned dilutions.

The conjugate for IFA test was "Wellcome" antihuman immunoglobulin in dilution 1/40 with 0.1% Evans-blue and for ELISA test, rabbit antihuman immunoglobulin labelled with alkaline phosphatase, prepared at the N.I.C.M., the Zoological Society of London, in dilution 1/800, was used.

IFA titers of 1/40 and over, and ELISA absorbance values (each sample was read on a Brinkmann colorimeter at 400 nm) 0.20 and over were considered as positive.

RESULTS

In Tables 1 and 2 the IFA results with *P. vivax* and *P. falciparum* malaria antigens are given. These results are expressed as positivity rates and geometric mean of reciprocal titres for the whole group and also for the serological positive group.

In Table 3 the ELISA results including positivity rates and the arithmetic mean titers of both whole group and serological positive group have been given.

In Table 4 comparison has been made between ELISA values and IFA titres of 652 serum samples tested with *P. falciparum* antigens. The relatively close results in both tests are shown inside double lined polygon which include 611 or 93.7% of the samples. In 32 (4.9%) cases the IFA titres and in 9 (1.4%) cases the ELISA values were considerably higher than the related malaria antibody level in other tests.

Table 5 gives the analysis of serological results in terms of blood films readings for malaria parasites.

Table 6 gives the results of parasitological examination and serological tests of 36 samples with *P. vivax*, *P. falciparum* and *P. fieldi* malaria antigens by IFA tests and *P. falciparum*, *P. fieldi* and mixture of *P. falciparum* *P. fieldi* malaria antigens by ELISA test.

DISCUSSION

The blood film results show that there is a low malaria parasitaemia detected by routine parasitological method in the areas investigated in southern Iran. But the serological tests, IFA and ELISA indicated much higher positivity rates with *P. falciparum* and especially with *P. vivax* malaria antigens. As stated in the earlier study⁽¹⁾ this may be due to the effect of wide distribution of antimalarial drugs which lowered the parasitaemia in submicroscopic numbers but did not prevent production of antibody. The results of each type of serological assay indicated a progressive increasing of positivity rate and antibody level with age. This has been demonstrated in earlier studies⁽¹⁾ and probably reflects both the greater response of older people and the higher challenge to which the older people would have been exposed to in the past.

The IFA showed a considerably higher positivity rate than the ELISA in the adults. This probably reflects the failings of all serological tests such as IHA, gel precipitin, as well as ELISA in which employ soluble malarial antigens. It seems that these tests do not detect such a complete spectrum of antibodies as the IFA which utilises whole parasites. This imposes a limitation on the value of procedures using soluble antigens, as they may under-estimate the malaria prevalence.

These tests may also fail to detect some individuals currently infected with malaria. In the present study several of the *P. vivax* cases were not positive by ELISA. Unfortunately a heterologous antigen was used because it was not practicable to prepare sufficient quantities of *P. vivax* soluble antigen. This again emphasises the necessity of carrying out blood film examination for parasites as well as serological evaluations in any epidemiological assessments of malaria.

The higher *P. vivax* parasite positivity rate and high IFA levels with *P. vivax* and *P. falciparum* malaria antigens in the 2-4 years age group may be due to a lower usage of preventive antimalarial drugs in this under school age group.

The small experiment using mixed *P. falciparum* and *P. fieldi* antigens in the ELISA test was not only economic in time and materials, but it was also more efficient in detecting higher titers of malarial antibodies. The use of such polyvalent antigens is to be recommended in serological surveys in malaria areas where more than one species of malaria parasite is present.

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TABLE 5
Serological results on the parasite positive (*P. vivax*) cases of the sera from Southern Iran

Age group	Total tested	Blood film +ves (<i>P. vivax</i>)	Serological results		
			ELISA +ves	IFA +ves	
				<i>P. vivax</i>	<i>P. falciparum</i>
2-4	42	2	0	2	0
5-9	139	3	1	3	3
10-16	127	2	1	2	2
17-30	146	3	0	3	3
over 30	198	2	1	2	1
Total	652	12	3	12	9

TABLE 6
ELISA Results on sera sample, tested with polyvalent *P. falciparum* and *P. fieldi* antigens

Age group	No. tested	Blood films +ves (<i>P. vivax</i>)	Mean ELISA Values (No. +ves > 0.2)			G.M.R. IFA titres (No. +ves \geq 1/40) <i>P. falciparum</i>
			<i>P. falciparum</i>	<i>P. fieldi</i>	<i>P. falciparum</i> & <i>P. fieldi</i>	
2-4	2	0	0.05(0)	0.06(0)	0.13(0)	0(0)
5-9	7	3	0.05(1)	0.06(0)	0.12(1)	24(4)
10-16	8	0	0.02(0)	0.05(0)	0.10(0)	0(0)
17-30	7	0	0.09(1)	0.08(0)	0.18(2)	36(3)
over 30	12	0	0.16(2)	0.11(3)	0.25(5)	60(7)
Total	36	3	0.09(4)	0.08(3)	0.17(8)	28(14)