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A Parasitological and Serological Study in Malaria Suspected Patients in Hormozgan Province, Southeastern Iran

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Abstract

Background: Presence of malaria immune factors induced by erythrocytic stages is widely used as an epidemiological approach to diagnose the infection mainly to distinguish the current, recent and past infections. This study was performed to find out the status of malaria, using microscopical and serological (IFA) methods in Bandar-Abbas and Minab, two malarious districts in Hormozgan Province of Iran.

Methods: 408 patients with suspected malaria symptoms were enrolled. Conventional microscopic examination and serological IFA test were employed for diagnosis of malaria. The rates of agreement between microscopical and serological diagnosis were analyzed by Kappa test.

Results: 17.9% and 1.7% of the samples were microscopically diagnosed as *P. vivax* and *P. falciparum,* respectively. On the other hand, the serum samples were sero-positive with *P. vivax* and *P. falciparum* antigens in 54.2% and 32.1% of the samples, respectively.

Conclusions: Serological IFA method could mainly determine the past history of malaria infection, but it was not helpful in detection of current infections. Moreover, there was no significant agreement between microscopical and serological (IFA) methods in diagnosis of malaria.

Keywords: Malaria; *Plasmodium vivax*; *Plasmodium falciparum*; Agreement; Microscopical; Serological; Iran

Introduction

Malaria parasites pass through a number of developmental stages, including both exoerythrocytic and erythrocytic forms within their vertebrate hosts. Each of these consists of a wide range of antigenic components which excite the response of the host's immune system. Such response usually results in at least a degree of acquired immuno-resistance to the disease. The presence of malaria immune factors induced by erythrocytic stages is widely used as an epidemiological approach to diagnose the infection,[1] mainly to distinguish the current, recent and past infections.[2] A few days after the blood is invaded by merozoites, antibodies to erythrocytic asexual parasites become detectable by sensitive serological tests. The antibodies reach a high level over one or two weeks and persist as long as the parasitaemia exists. At last, antibodies fall back to a lower level,[3] and if the infection remains at the subpatent level for a long time, the antibody may still be detectable for many months or years even after clearance of parasitaemia.[4] However,

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serological and microscopical examinations are principal tools for understanding epidemiology and control of malaria at a community level. A cross-sectional serological and microscopical survey of malaria in Bandar-Abbas and Minab, the two malarious districts of Hormozgan Province in southeast of Iran was conducted to find out the present parasitological and serological status of malaria in these regions. According to the report of Center for Diseases Management (CDM) in Iran, 19285 cases of malaria were microscopically diagnosed in 2006 in Iran, about 88%, 11% and 1% of which were detected as *plasmodium vivax, P. falciparum* and *mixed* infections, respectively. At the same time, 4958 malaria cases with 98.1% *P. vivax,* 1.8% *P. falciparum* and 0.1% *mixed* infection were recorded in Hormozgan Province.

This survey, to our knowledge, is the second study of its type in Iran. The first study was conducted in the north, north-west and south-west areas of Iran during 1975-1982.[5]

Materials and Methods

The study was undertaken from February 2006 to December 2006 at Bandar-Abbas and Minab districts in Hormozgan Province located at the southeastern Iran. The weather in these districts is warm and humid with minimum and maximum temperatures of about 27.5^0C and 33^oC, respectively (average 30.2^oC) in malaria transmission seasons. Two to three health centers (according to the records of *vivax* and *falciparum* malaria during the last two years) as passive sentinel sites and four villages for random sampling were selected in each district. *Plasmodium vivax* is a predominant species in the studied areas and *plasmodium falciparum* still remains the second species of *plasmodia* in the province. The most important active vectors considered are *Anopheles stephensi*, *Anopheles culicifacies* and *Anopheles fluviatilis.* According to the report of Centre for Diseases Management, the annual parasite incidence (API) in the studied areas was 5 in 2006.

A total of 408 individuals (206 from Bandar-Abbas and 202 from Minab districts) that were suspected to having malaria symptoms were enrolled. There were 262 male and 146 female subjects with an age range of 1-87 years (average 44 years). The sample size was estimated based on 3% disagreement between serological and microscopical diagnosis of *P. vivax* and *P. falciparum,* 95% confidence level and 80% power.[12] Blood samples, obtained from recruited individuals via finger prick, were collected into micro-tubes and then the relevant sera were separated and kept frozen. Prior to admission, an informed consent was obtained from each patient or his/her guardians.

Microscopical examination: The required information of patients such as biodata and the results of microscopically examined Giemsa stained thick blood films were recorded on the relevant forms. A 5-ml infected blood with either *P. vivax* or *P. falciparum* taken from a number of malaria patients with high parasitaemia was collected onto glass slides for preparing antigens using a hemobile instrument.[6,7] The slides were covered with water proof bags and were kept in freezer of -70 ^{0}C until use.

Serological tests: The antibodies of the collected sera and dried blood samples on filter papers were measured by indirect fluorescent antibody (IFA) test according to the method described by Voller and O'Neill.[7]

Briefly, the packets of test slides containing antigens were removed from the freezer and allowed to warm in room temperature before being unwrapped. Each spot of the antigens was rounded by a permanent pen for the next process. The slides were then fitted with face upwards into the appropriate trays. Serum dilutions were prepared in 96-well microtiter plates starting from 1:10 and ending to 1:2560 with 1:20, 1:40, 1:80, 1:160, 1:320, 1:640 and 1:1280 dilutions between them. Then, the dilutions were transferred onto the antigens by means of micropipettes. The subsequent processes for performance of the indirect fluorescent antibody test were as follows: incubation of the Ag + Ab slides in a humid chamber for 30 minutes, washing the slides with PBS (Phosphate-buffer saline) for 7 minutes about 3 times, applying fluorescein-labelled anti-human conjugates

(DAKO Co., with 0.1% Evans blue stain) to each spot of the slides and reincubating in the humid chamber for 30 minutes, rewashing the slides for three times by PBS, applying a few drops of glycerol–buffer on the spots, covering them with a cover slip and eventually examining the spots under the fluorescent microscope.

The spots were read under 650 x magnification of Leitz fluorescent microscope. Reading commenced with the strongest serum dilution and proceeded to the progressively weaker one. For each set of the test, positive serums of *P. vivax* and *P. falciparum* and a negative serum were used as the positive and negative

controls. Moreover, the geometric mean of reciprocal titers (GMRT) for sero-positive cases was calculated.[5] The rate of agreement between microscopical and serological diagnoses was analyzed by Kappa test.[8]

Results

The indirect fluorescent antibody (IFA) responses and microscopical findings of the collected samples for *P. vivax* and *P. falciparum* are summarized in Tables 1-4.

The results obtained from conventional microscopical method in Bandar-Abbas district showed that 19.4% and 1.5% of the cases were diagnosed as *P. vivax* and *P. falciparum* respectively. In contrast, 47.1% and 26.7% of the cases were seropositive with *P. vivax* and *P. falciparum* antigens in titers≥1:20, respectively (Table 1 and 2). Moreover, in Minab district, 16.3% and 2% of the studied samples were diagnosed as *P. vivax* and *P. falciparum* using microscopical method, but in serological detection 61.4% and 37.6% of the samples were seropositive with *vivax* and *falciparum* antigens in titers≥1:20, respectively (Table 1 and 2). Comparative results for the both *plasmodia* with the relevant rate of Kappa and *p values* can be found in Table 3 for the whole studied areas.

The total IFA responses and microscopical results of the collected samples for the whole studied areas

Table 1: Comparative illustration of microscopical and serological data for *Plasmodium vivax* in Bandar-Abbas and Minab districts (Hormozgan Province, Iran, 2006)

District	Microscopical Results	IFA serological results		Total No (%)	GMRT	Statistical test
		< 1:20 No (%)	≥ 1:20 No (%)			
Bandar-Abbas	Negative	102 (49.5)	64 (31.1)	166 (80.6)	67	Kappa=0.28 *p*<0.001
	Positive	7 (3.4)	33 (16.0)	40 (19.4)	334.4	
	Total	109 (52.9)	97 (47.1)	206 (100)	126.2	
Minab	Negative	76 (37.6)	93 (46.1)	169 (83.7)	110.5	
	Positive	2 (1.0)	31 (15.3)	33 (16.3)	501.2	Kappa=0.18 *P*<0.001
	Total	78 (38.6)	124 (61.4)	202 (100)	165	

Table 2: Comparative illustration of microscopical and serological data for *Plasmodium falciparum* in Bandar-Abbas and Minab districts (Hormozgan Province, Iran , 2006)

District	Microscopical Results	IFA serological results		Total	GMRT	Statistical test
		<1:20 No (%)	≥1:20 No (%)	No (%)		
Bandar-Abbas	Negative	151 (73.3)	52 (25.2)	203 (98.5)	38.2	Kappa=0.07 *P*<0.001
	Positive	0 (0.0)	3 (1.5)	3 (1.5)	100	
	Total	151 (73.3)	55 (26.7)	206 (100)	41.2	
Minab	Negative	126 (62.4)	72 (35.6)	198 (98.0)	65.2	Kappa=0.06 *P*<0.001
	Positive	0 (0.0)	4 (2.0)	4 (2.0)	631	
	Total	126 (62.4%)	76 (37.6%)	202 (100%)	77.3	

Table 3: Microscopical and serological results of *P. falciparum* and *P. vivax* diagnosis in Bandar-Abbas and Minab districts (Hormozgan Province, Iran, 2006)

Malaria parasite	Microscopic Results	IFA serological results		Total No (%)	GMRT	Statistical test
		<1:20 No (%)	≥1:20 No (%)			
Plasmodium falciparum	Negative	277 (67.9)	124 (30.4)	401 (98.3)	52.4	
	Positive	0 (0.0)	7 (1.7)	7 (1.7)	316.2	Kappa=0.07 *p*<0.001
	Total	277 (67.9)	131 (32.1)	408 (100)	60	
Plasmodium vivax	Negative	178 (43.6)	157 (38.5)	335 (82.1)	91.3	
	Positive	9 (2.2)	64 (15.7)	73 (17.9)	398.1	Kappa=0.23 *p*<0.001
	Total	187 (45.8)	221 (54.2)	408 (100)	146.3	

Table 4: Comparative illustration of microscopical and serological diagnosis for *P. falciparum* and *P. vivax* according to age group in Bandar-Abbas and Minab districts (Hormozgan Province, Iran, 2006)

Age groups (years)	Microscopical results				Serological results					
					Plasmodium vivax			Plasmodium falciparum		
	Neg. No (%)	P. v* No (%)	P. f** No (%)	Total No (%)	<1:20 No (%)	≥1:20 No (%)	Total No (%)	<1:20 No (%)	≥1:20 No (%)	Total No (%)
1-6	21 (77.8)	5 (18.5)	1 (3.7)	27 (100)	11 (40.7)	16 (59.3)	27 (100	20 (74.1)	7 (25.9)	27 (100)
7-18	86 (77.5)	23 (20.7)	2 (1.8)	111 (100)	55 (49.5)	56 (50.5)	111 (100)	73 (65.8)	38 (34.2)	111 (100)
19-35	138 (83.6)	24 (14.5)	3 (1.8)	165 (100)	75 (45.5)	90 (54.5)	165 (100)	114 (69.1)	51 (30.9)	165 (100)
>35	83 (79.0)	21 (20.0)	1 (1.0)	105 (100)	46 (43.8)	59 (56.3)	105 (100)	70 (66.7)	35 (33.3)	105 (100)
Total	328 (80.4)	73 (17.9)	7 (1.7)	408 (100)	187 (45.8)	221 (54.2)	408 (100)	277 (67.9)	131 (32.1)	408 (100)
Statistical test	P (Fisher Exact test)=0. 67				x^2 = 1.08 df = 3 *p*=0.78			x^2 = 0.88 df =3 *p*=0.83		

**Plasmodium vivax, **Plasmodium falciparum*

are summarized by age group in Table 4. In our study, the Kappa values ranging from 0.06 to 0.28 indicate a weak agreement between the above- mentioned methods.

Discussion

Although conventional light microscopy is still the standard method for diagnosis of malaria infection at most of the malarious areas, the method may yield negative results in case of the lack of parasites in the peripheral blood or in the low parasitaemia situation.[9] On the other hand, about one week after the parasites undergo erythrocytic schizogony, the relevant antibodies can be detected in the blood by specific serological tests. The antibody level persists as long as parasitological crisis is obvious. Eventually, the level of antibody falls back to a lower level.[3] There is no doubt that both microscopical and serological methods can be used for detection of malaria infection but in the different scopes. The wide variation between

individuals, plasmodia species and strains makes, more or less, different interpretations for the acquired results of microscopical and serological studies. This study was carried out to detect malaria infection in individuals with suspected malaria symptoms by using microscopical and serological (IFA) methods comparatively. Microscopic diagnosis as usual indicated the parasite-detectable infections, but not seropositive cases in which parasites may stay in subpatene level. It is important to differentiate active seropositive infections from the non-active cases, particularly owing to the potential transmission possibility of malaria in the endemic areas. The results obtained in this study showed that a considerable number of the studied population was exposed to malaria infection especially in Minab district.

According to the microscopical and IFA results obtained from 408 samples including thick blood films and serum and with the intention of kappa test, there was no significant agreement between microscopical and serological methods in Bandar-Abbas and Minab districts. The results of an extensive serological study conducted by Edrissian and his colleagues[5] in north, northwest and southwest of Iran reflected usefulness of IFA method for epidemiological surveys and endemicity of malaria. Similar results were also reported by other investigators from different parts of malarious areas in the world.[10,11]

Age related results showed that the prevalence of antibodies detectable *P. falciparum* antigen was more common in the older age groups, but such prevalence with *P. vivax* antigen was observed in all age groups (Table 4). This result indicated that persistence and prevalence of *P. vivax* was more than *P. falciparum* in the studied areas.

In conclusion, this study showed that the serological (IFA) method could be useful as a tool for determination of the history of the infection and epidemiological study of malaria, but not for early and actual detection of the disease, and also there was no significant agreement between microscopical and serological (IFA) methods in the diagnosis of malaria.

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