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MALARIAL ANTIBODIES AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G-6-PD)DEFICIENCY

Gh.H.Edrissian Pharm.D.,D.C.L.S., MSC., K.Montazemi, Pharm.D.,D.C.L.S., A.R. Nasseri, D.V.M. and A.Afshar.*

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ABSTRACT

The IFA and the blue dye decolourization G-6-pD tests were applied in three cross-sectional studies to find out the relationship of malarial antibodies and G-6-pD deficiency in the male residents of the malarious areas of southern Iran.

In the first study that the blood samples were collected in a random sampling method from the whole Hormozgan province, the G-6-PD defieint individuals had,significantly, lower sero-positive rate (SPR) and also,considerably lower total geometric mean of reciprocal titres (GMRT) with P.falciparum antigen as compred to the G-6-PD normal subjects. But with P.vivax antigen SPR and GMRT in both groups were almost the same.

However in the second and third studies that the blood samples were collected from the selected groups of the residents of the above Hormozgan province with high incidences of malaria no such distinct serological differnces between G-6-PD deficient and G-6-PD normal groups was observed.

It is concluded that the cross-sectional serological survey of malaria in populations who are more frequently

* *School of Public Health and Institute of Public Health Research,Teheran University,P.O.Box 3918-11365,Teheran, Iran.*

exposed to malarial infections, particularly in the areas where P.vivax is also prevalent is not enough to show a conclusive serological evidence to support G-6-PD/malaria hypothesis.

INTRODUCTION

The hypothesis that the carriers of G-6-PD deficiency trait have relative natural resistance to infection with Plasmodium falciparum was put forward by Motulky and also-Allison both in 1960.(34,1). These authors and later some others(40,11,15,42,4,41,5,43,22)found intresting positive correlation between the frequencies of G-6-PD deficiency and status of malarial endemicities even in the areas where malaria was endimic in the past.

The suggestion of innate relative resistance to malignant malaria in the enzyme deficient subjects has been supported by: low parasite rate and density(2,17,20 , 29), lower mortality rate(34,18), lower-percentage of G-6-PD deficient parasitized eryrhrocytes than the percentage of parasite contining cells with normal enzyme(27), lower G-6-PD deficiency rate among patients with malaria parasitaemia as compared to the rate in subjects free from malrial infection or patients with other infections(18 ,10) and finally less efficient growth of P.falciparum parasites in G-6-PD deficient erythrocytes in malaria patients (25) or in the in-vitro culture of P.falciparum in presence of an oxidant stress(16).

However, there are also considerable number of studies with the results that are in conflict with the above supporting evidences such as, the lack of significant differences of parasite rates and densities(26,38, 36,6,7) and also resistance against severe falciparum infection(26,39 ,28,31) between enzyme deficient and normal studied subjects.

Some investigators are doubted whether there are at least in some areas, satisfactory evidences available to support the hypothesis that G-6-PD deficiency confers a biological advantage against malaria(31, 9,30) and some other are believed that sinergistic interaction of other factors such as thalassaemia(41,19), viral infection,dietary habits and social customs(24), favism(23,8) and also other oxidant stress(16,32) play a role in G-6-PD/malaria

hypothesis.

As the serological survey of malaria gives informations about the total experience of malaria of the individuals in a community(13), in the present investigation indirect fluorescent antibody(IFA)technique has been applied to find the possible relationship of malarial antibodies and G-6-PD deficiency in the residents of the malarious areas of Hormozgan province in southern Iran.

Materials-and Methods

Study area and its malaria status

Hormozgan province is located in southern Iran on the northern coast of Persian Gulf and Oman Sea . It has a tropical climate with average temperature and relative humidity ranging 12-50 C and 40-80% respectively.

Malaria was hyper-endemic in this area up to 1950 . Afterwards, the anti-malarial campaign including residual spraying, antilarval measures and mass drug distribution reduced, considerably, the malaria incidence.Nevertheless the transmission of the disease due to technical and operational provlems is still going on with various rates in different parts of this province (33) . The prevalent plasmodia in the area are P.vivax and P.falciparum.

Sampling

The blood samples were collected in Hormozgan province in three different occasions (or three studies) as follows:

Samples A) From the samples collected in a random sampling method from the residents of Hormozgan province in a Health Survey Project , carried out by the School of PH&IPHR (Jan.-Mar.,1975) 297 Samples belonging to the male subjects.

Samples B) From the blood samples collected during Aug. 1977 from the majority of the residents of Gohreh village,Bandar-Abbas area, (where the incidence of malaria is rather high) and also from the outpatients of Malaria Laboratory , Bandar-Abbas Malaria Unit, respectively 140 and 141 samples belonging to the male subjects.

Samples C) From the male children(1.5 to 14 years old)of the residents of Berentin village.Minab area, where an outbreak of malaria (mostly falciparum malaria) was occured in 1980, 144 samples collected in Aprill 1981.

Blood examinations

All collected blood samples were examined by the following procedures:

1- The routine microscopical examination of the Giemsa stained thick blood films for malaria parasites.

2- The visual blue dye decolourization G - 6- PD test based on the method described by Motulsky and Campbell-Kraut 1961 (35).using Sigma Kit, was applied for detection of G-6-PD deficiency in the blood samples collected in the tubes containing EDTA anticoagulant (Samples A) or in heparinzed capillary tubes (Samples B & C).

3- The indirect fluorescent antibody testing (IFAT) of collected serum samples (samples A) for determination of malarial antibodies with Aotus P.falciparum and P.vivax malaria antigens (received from NICM, the Zoological Society of London) or plasma samples(Samples B&C) with human P.falciparum and P.vivax malaria antigens,prepared from malaria patients in Bandar-Abbas Research Station was carried out as the method described by Voller & O'Neill., 1971(44).

Results

Results of examinations of the blood samples (A,B&C) belonging to the male subjects in the three different studies were as follows:

Samples A 1- In the microscopical examination of the297 thick blood films of Hormozgan province four cases (1.3%) of P.vivax (in G- 6 - PD normal subjects) were observed.

2- In the blue dye decolourization G- 6 - PD test, the enzyme deficiency were detected in 53 (17.8%) subjects.
3- In the IFAT of the serum samples, malarial antibodies were determined in titres 1:20 to 1:1280 with Aotus P.falciparum and 1:20 to 1:2560 with Aotus P.vivax antigens , in 107 (36.0%) and 195 (65.6%) cases,respectively.

Samples B 1- In the microscopical examination of the140 thick blood films of Gohreh village 5cases (3.5%)of P.vivax (3 cases in G-6-PD normal and 2 cases in G-6-PD deficient subjects) were observed.
Among 141 out-patients 11 (7.8%) cases of P.falciparum (8 cases in G-6-PD normal and 3 cases in G-6-PD deficient subjects)and35 (24.8%) cases of P.vivax (32 cases in G-6-PD normal and 3 cases in G-6-PD deficient subjects) were detected.
2- The enzyme deficiency in the residents of Gohreh village and in out-patients were 32 (22.8%) and 27 (19.1%) cases respectively.
3- In the IFAT of the plasma samples,malarial antibodies were determined in titres 1:20 to 1:2560 with human P.falciparum and P. vivax antigens, in 95 (67.8%)and119(85.0%) cases in Gohreh village and 72(51.0%) and 95(67.3%) cases in malaria suspected outpatients, respectively.

Samples C 1- In the microscopical examination of the144 thick blood films collected from children in Berentin village 5 cases (3.4%) of P. vivax (3 cases in G-6-PD normal and 2cases in G-6-PD deficient subjects) were observed.
2- The enzyme deficiency was detected in 27 (18.7%) subjects.
3- In the IFAT of the plasma samples malarial antibodies were determined in titres 1:20 to 1:1280 with human P.falciparum and 1:20

to 1:320 with human P.vivax antigens, in 58 (40.2%) and 39 (27.0%) cases respectively.

Sero-positive rates (SPR) and total geometric means of reciprocal titres (GMRT) with P.falciprum and P.vivax antigens in G-6-PD normal and G-6-PD deficient subjects in relation to their ages for the three studies are given in Tables 1 to 4.

In the first study (samples A) SPR with P.falciparum antigen in the enzyme deficient subjects was significantly ($x^2 = 5.01$, $0.02 > P > 0.0.1$) lower than the SPR in G-6-PD normal subjects. The total GMRT was also considerably lower in G-6-PD deficient subjects. However, with P.vivax antigen in this study SPR and GMRT in G-6-PD deficient and G-6-PD normal subjects were almost the same.

In the second study (Samples B) in Gohreh village the total GMRT with both P. falciparum and P.vivax antigen in G-6-PD deficient individuals were considerably higher than the total GMRT in G-6-PD normal subjects.

The other obtained serological data in second (Samples B) and also third (Samples C) studies do not show such significant differences.

SPR and GMRT with both P.falciparum and P.vivax antigens in the enzyme deficient individuals as weel as in the G-6-PD normal subjects are more or less, increasing with age, but there is no distinct correlation between G-6-PD deficiency frequencies in different age groups in this present investigation.

Table,1.

IFA P.falciparum and P.vivax antibodies in G-6-PD normal and G-6-PD deficient male subjects in Hormozgan Province,Southern Iran(1975).

Age group (years)	G-6-PD Normal							G-6-PD Deficient						
	No. examined	P.falciparum			P.vivax			No. examined	P.falciparum			P.vivax		
		No. Pos. ≥1:20	% Pos. (SPR)	GMRT*	No. Pos. ≥1:20	% Pos (SPR)	GMRT*		No. Pos. ≥1:20	% Pos. (SPR)	GMRT*	No. Pos. ≥1:20	% Pos. (SPR)	GMRT*
2-4	20	3	15.0	1.6	4	20.0	2.4	0	0	0	0	0	0	0
5-9	60	10	16.6	1.9	33	55.0	9.6	16	1	6.2	1.2	8	50.0	7.5
10-19	55	9	16.3	1.8	26	47.2	6.9	11	1	9.0	1.3	7	63.6	9.2
20-39	53	29	54.7	8.3	44	83.0	39.3	10	3	30.0	3.1	8	80.0	17.8
≥40	56	44	78.5	27.4	51	91.0	62.3	16	7	43.7	10.0	14	87.5	52.3
Total	244	95	38.9	4.7	158	64.7	16.7	53	12	22.6	2.7	37	69.8	16.6

* *GMRT : Total Geometric Mean of Reciprocal Titres*

Table 2,

IFA P.falciparum and P.vivax antibodies in G-6-PD normal and G-6-PD deficient male subjects in Gohreh village, Bandar-Abbas area, Hormozgan province, Southern Iran (1977).

Age group (years)	G-6-PD Normal							G-6-PD Deficient						
	No. examined	P.falciparum			P.vivax			No. examined	P.falciparum			P.vivax		
		No. Pos. ≥1:20	% Pos. (SPR)	GMRT*	No. Pos. ≥1:20	% Pos. (SPR)	GMRT*		No. Pos. ≥1:20	% Pos. (SPR)	GMRT*	No. Pos. ≥1:20	% Pos. (SPR)	GMRT*
1-4	20	4	25.0	1.9	14	70.0	14.6	3	2	66.6	18.5	2	66.0	18.5
5-9	33	22	66.6	10.1	29	87.8	31.4	6	3	50.0	5.6	5	83.8	24.2
10-19	23	14	60.8	13.1	19	82.6	29.2	11	8	72.7	22.6	9	81.8	55.6
20-39	10	9	90.0	72.5	9	90.0	83.2	5	4	80.0	25.3	5	100	276.0
≥40	22	22	100	358.0	20	90.9	137.0	7	7	100	425.0	7	100	107.0
Total	108	71	65.7	20.3	91	84.2	39.7	32	24	75.0	33.1	28	87.5	61.3

* *GMRT : Total Geometric Mean of Reciprocal Titres*

Table,3.

IFA P.falxiparum and P.vivax antibodies in G-6-PD normal and G-6-PD deficient male malaria suspected out-patients in Bandar-Abbas city,Hormozgan province, Southern Iran(1977).

Age group (years)	G-6-PD Normal							G-6-PD Dificient						
	No. examined	P.falciparum			P.vivax			No. examined	P.falciparum			P.vivax		
		No. Pos. ≥1:20	% Pos. (SPR)	GMRT*	No. Pos. ≥1:20	% Pos (SPR)	GMRT*		No. Pos. ≥1:20	% Pos. (SPR)	GMRT*	No. Pos. ≥1:20	% Pos. (SPR)	GMRT*
2-4	5	2	40.0	3.8	2	40.0	3.4	2	0	0	0	1	50.0	20
5-9	17	5	29.4	2.7	9	52.9	17.2	4	2	50.0	15.0	4	100	94.6
10-19	45	19	42.2	7.7	27	60.0	22.6	11	3	27.2	6.1	9	81.8	19.8
20-39	38	27	71.0	21.2	27	71.0	50.7	6	5	83.8	38.3	3	50.0	10.3
≥40	9	5	55.5	3.8	9	100	69.9	4	4	100	103.4	4	100	47.3
Total	114	58	50.8	9.1	74	64.9	28.6	27	14	51.8	14.5	21	77.7	22.4

* *GMRT : Total Geometric Mean of Reciprocal Titres*

Table,4.

IFA P.falciparum and P.vivax antibodies in G-6-PD normal and G-6-PD deficient male children in Berentin village,Minab area,Hormozgan Province,Southern Iran(1981).

Age group (years)	G-6- Normal							G-6-PD Deficient						
	No. examined	P.falciparum			P.vivax			No. examined	P.falciparum			P.vivax		
		No. Pos. ≥1:20	% Pos. (SPR)	GMRT*	No. Pos. ≥1:20	% Pos. (SPR)	GMRT*		No. Pos. ≥1:20	% Pos. (SPR)	GMRT*	No. Pos. ≥1:20	% Pos. (SPR)	GMRT*
1.5-4	23	2	9.5	1.4	5	21.7	2.3	5	1	20.0	2.0	1	20.0	3.1
5-9	48	13	27.8	2.9	11	22.9	2.5	11	5	45.4	5.0	3	27.2	3.7
10-14	46	30	65.2	19.6	16	34.7	3.8	11	7	63.6	19.5	3	27.2	2.5
Total	117	45	38.4	5.3	32	27.3	2.9	27	13	48.1	7.4	7	25.9	3.1

* *GMRT: Total Geometric Mean of Reciprocal Titres*

Discussion

A lot of studies, mostly conculded or based on epidemiological, clinical and parasitological observations have been carried out on the hypothesis that, the carriers of G-6-PD deficiency trait have a relative natural resistance to falciparum infection since 1960 that this hypothesis was suggested by Motulsky and also Allison(34,1).

In the present investigation the serological IFAT was used for the first time to study the relationship between malarial antibodies and G-6-PD deficiency in the male residents of the malarious areas of southern Iran.

Generally, the frequency of the enzyme deficiency in the male subjects in the studied malarious areas were considerably high (17.8-22.8%) as compared to the rates reported for some other parts of Iran (4,5,21) . In this present study the highest enzyme deficiency rate(22,8%) found among the residents of Ghoreh village who had also the highest seropositive rate (SPR) and the total geometric mean of reciprocal titres (GMRT) with both P . vivax and P.falciparum antigens.

The parasitological findings in the studied areas , which are occasionally under irregular mass drug distribution programmes , as found previously in a serological and parasitological studies of malaria in this part of Iran (14), did not show the real status of malaria as it is estimated from the obtained serological data.

In comparative serological studies of malaria in G-6-PD deficient and G-6-PD normal subjects in the first study that the blood samples were collected in random sampling method from the male residents of the whole Hormozgan province the G-6-PD deficient subjects had, significantly($P<0.05$),lower SPR with P.falciparum antigen and the total GMRT was also lower. but with P.vivax antigen SPR and GMRT in both G-6-PD normal and G-6-PD deficient subjects were almost the same. However, in the second and third studies that the blood samples were collected from the specially selected groups of the male residents of the above studied province,with rather high incidences of malaria,there was no signicicant difference between SPR in G-6-PD deficient and G-6-PD normal subjects neither with P.falciparum nor with P.vivax antigens. Total GMRT in the residents of Gohreh village in the cases of both P.falci-

parum and P.vivax antigens in G-6-PD deficients were even considerably higher in compare to G-6-PD normal subjects.

The explanation for the obtained conflicting results could be as follows:

The lower density of P.falciparum parasites in G-6-PD deficient individuals (2,17) and less efficient growth of the parasites in the enzyme defficient erythrocytes (25) may cause the less efficiency, in G-6-PD deficient individuals, in producing and maintaining P.falciparum malaria antibody. Therefore in the whole Hormozgan province where the total indidence of malaria is low , the serological data have indicated that G-6-PD deficient subjects had totally less experiences with falciparum infection.Consequently, the relative natural resistance of the enzyme deficient individuals to falciparum infection have been serologically approved in the first study. However in the selected groups of the above population who were more frequently exposed to malaria infections, the cross sectional serological surveys of malaria could not show the possible exist differences between P.falciparum serological data in G-6-PD deficient and G-6-PD normal groups examined in the second and third studies.

More frequent deterioration of the enzyme deficient parasite containing erythrocytes and liberation and death of the parasites (15,17)may be and explanation for the elevation of GMRT in the enzyme deficient individuals in Gohreh Village during active transmission season.

Although, generally, the results of the present investigation more or less support G-6-PD/malaria hypothesis, further longitudinal serological studies of malaria in G-6-PD deficient and G-6-PD normal residents of malarious areas, preferably in the areas where P.vivax is not so prevalent (3) as it was in our studied areas, may conclusively clear cut and most probably approve the G-6-PD malaria hypothesis.

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