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Investigation of Ahaptoglobinemia and its Association with Malaria Endemicity in South of Iran

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Abstract

Ahaptoglobinemia is a common phenomenon in tropical countries,where it is probably due to malaria-induced haemolysis. Previous studies have suggested that ahaptoglobinemia is more useful than the other parasite detection indexes to estimate the endemicity of malaria. The present study shows the relationship between malaria and haptoglobin, as a genetic marker in Balouch population of Sistan & Baluchistan provience, the highly malaria endemic area in southeast of Iran. Using starch gel electrophoresis, the results showed 20.2% ahaptoglobinemia in patients (n=203) and 3% in controls (n=197). Statistical analysis showed significant association between ahaptoglobinemia and malaria (P<0.001).Therefor, the frequency of ahaptoglobinemia may be more than the frequencies obtained from blood smear studies (1.9%) in malariuos patients.

Keywords: *Malaria, Genetic markers, Haptoglobin, Ahaptoglobinemia, Balouch Population, Iran*

Introduction

Malaria is a primary cause of mortality and morbidity especially in children living in tropical areas, killing about 2 million people per year (1). Malaria control and/or eradication programs require the assessment of the state of malaria in defined areas. The most commonly used methods to assess malaria, are clinical symptoms such as fever, determination of the spleen size in children, examination of periperal blood smear and measurment of antimalaria antibodies (2). Each of these methods presents some limitations such as insensitivity (blood smear) and lack of specificity (spleen size) (2). Investigations show that measurment of the serum concentration of haptoglobin could be used as an indicator for the determination of malaria endemicity (2). Haptoglobin, a serum protein of the alpha–2 fraction, binds to free haemoglobin whenever intravascular haemolysis occurs. The resulting complex is then cleared by the mononuclear-phagocyte system. Haptoglobin disappears from the plasma, leading to hypohaptoglobinemia or ahaptoglobinemia. 10-40% of people in some tropical areas have very low serum haptoglobin levels (3). Several investigations have shown that the absence of haptoglobin may be linked to malaria –induced haemolysis rather than, as had previously been postulated, to the existence of a haptoglobin O phenotype. There is polymorphism in haptoglobin gene .Two common allels, HP1 and HP2 exist in this polymorphic system, and so three phenotypes, HP1-1, HP2-1 and HP2-2. Normally about 2% of populations are genetically ahaptoglobinemic. No alteration was found in most of ahaptoglobinemics DNA studies (4). It was shown that ahaptoglobinemia was suppressed within a few weeks by antimalaria chemoprophylaxis (5).

Materials and Methods

A total of 203 highly susceptible patients with history of several times of malaria infections and 197 healthy controls with no history of malaria infection were studied in Baluch population of Sistan & Baluchistan, a highly malaria endemic area in southeast of Iran. Periperal blood samples were collected and blood smears were prepared. For the cases

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that were infected in time of sampling (n=91), the type of parasite were determined (52 Plasmodium *vivax* and 39 *Plasmodium falsiparum*). Starch gel electrophoresis (6), were used to find Hp phenotypes (Figure 1). Statistical analysis, by using the Woolf's test (7), showed significant results in some parameters.

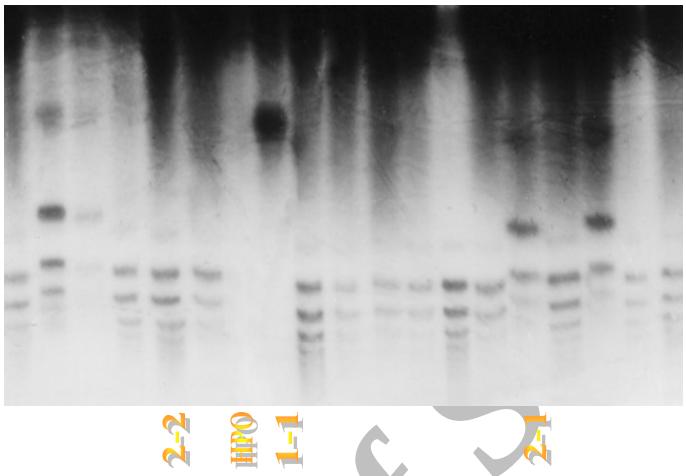

Fig. 1: Haptoglobin patterns (2-2, 2-1, 1-1 and HPO) by using starch gel electrophoresis method

Results

The phenotype frequencies are shown in table 1. No band was observed in electrophoretic patterns of 41 patients (20.2%) and 6 controls (3%). This difference is statistically significant (P<0.001). of course, according to the previous studies, most of these cases are not real ahaptoglobinemia. So, they were excluded in other statistic analysis as shown in table 2. The results showed no significant difference.

Table 1: Haptoglobin phenotype frequencies (including HPO) in 203 patients with malaria & 197 controls

Phenotypes	**Patients**		**controls**		**Woolf's test indexes**		
	Number	Percent	Number	Percent	RI	X^2	P
1-1	10	4.9	20	10	0.4585	3.3708	N.S
2-1	68	33.5	66	33.5	0.9997	0.0000	N.S
2-2	84	41.4	105	53.5	0.6185	5.6715	0.03>P>0.01
O	41	20.2	6	3	8.0566	21.5021	0.001>P
Total	203	100	197	100			

N.S = Not Significant

Table 2: Haptoglobin phenotype frequencies (excluding HPO) in 162 patients with malaria & 191 controls

Phenotypes	**Patients**		**controls**		**Woolf's test indexes**		
	Number	Percent	Number	Percent	RI	X^2	P
1-1	10	6.2	20	10.5	0.5625	2.038	N.S
2-1	68	41.9	66	34.5	1.37	2.0439	N.S
2-2	84	51.9	105	55	0.8820	0.3436	N.S
Total	162	100	191	100			

N.S = Not Significant

The association between the type of parasite, *Plasmodium falciparum* and *Plasmodium vivax* with haptoglobin phenotypes was studied too. As is shown in table 3, 17(43.6%) of patients infected with *P.falciparum* and 11(21.2%) of patients infected with *P.vivax* were ahaptoglobinemic. Patients with Hp2-1 phenotype were more infected with *P.vivax* 24(46.2%), than *P.falciparum* 10(25.6%). Excluding HPO showed no significant difference between *P.vivax* and *P.falciparum* with HP phenotypes (Table 4).

Table 3: Haptoglobin phenotype frequencies (including HPO) in 52 patients with *P.vivax* and 39 patients with *P.falciparum*

Phenotypes	***P.vivax***		***P.falciparum***		**Woolf's test indexes**		
	Number	Percent	Number	Percent	RI	X^2	*P*
1-1	2	3.9	1	2.6	0.6579	0.1133	N.S
2-1	24	46.2	10	25.6	0.4023	3.9134	0.05>P>0.02
2-2	17	33.7	11	28.2	0.8090	0.2099	N.S
O	11	21.2	17	43.6	2.8801	5.0961	0.03>P>0.01
Total	52	100	39	100			

N.S = Not Significant

Table 4: Haptoglobin phenotype frequencies (excluding HPO) in 43 patients with *P.Vivax* and 22 patients with *P.Falciparum*

Phenotypes	***P.vivax***		***P.falciparum***		**Woolf's test indexes**		
	Number	Percent	Number	Percent	RI	X^2	P
1-1	2	5	1	4.6	0.9762	0.0003	N.S
2-1	24	56	10	45.4	0.6597	0.6232	N.S
2-2	17	39	11	50	1.5294	0.6468	N.S
Total	43	100	22	100			

N.S = Not Significant

Discussion

This is the first report on ahaptoglobinemia in malarious patients in Iran. Previous studies reported that 95% of individuals with ahaptoglobinemia or hypohaptoglobinemia returned to normal level of haptoglobin after treatment for malaria in other hyperendemic areas (2). It was also shown that no gene deletion or rearrangement occurred using gene mapping in ahaptoglobinemics(4). Level of serum haptoglobin in malaria patients depends on the level of parasitemia or severity of infection.Increasing haemolysis cause increase of free serum haemoglobin. Haptoglobin as a haemoglobin binding protein binds to it and activated reticuloendotelial system absorbs it and induces rapid prossessing of Hb-Hp complex, finally the complex excludes from plasma. According to the level of haemolysis, level of haptoglobin could vary so patients with low parasitemia shows hypohaptoglobinemia and those with severe haemolysis show ahaptoglobinemia.

The amount of haptoglobin can be measured by techniques such as ELISA (8) and immunonephelometry (5). In the present study, at first it was intended to study the association between malaria and different phenotypes of haptoglobin as a genetic marker. Haptoglobin had shown significant association with some diseases such as hepatitis, cirrhosis (9), etc. In some cases, HP bands in starch gel electrophoresis were very light which could be due to low concentration of haptoglobin or hypohaptoglobinemia. Out of 203 patients, 41(20.2%) cases had no detectable band which means no haptoglobin were present in their serum (ahaptoglobinemia). So this finding suggests 20.2% endemicity of malaria in Baluch population of Iran, whereas the positive blood smears which were reported by health centers for this area previously, was

1.9%.The amount of ahoptoglobinemai in central Africa was reported as 30%(10). Because of higher sensitivity of haptoglobin index than blood smear, the real prevalence of malaria must be close to our results. The cause of 6% ahaptoglobinemics in control group which had no history of malaria infection in their lives may be other haemolitic diseases such as G6PD deficiency (11) or lack of haptoglobin gene. Some of the patients had detectable parasite in blood smear in time of sampling. The others had history of at least 5 times infection, with no positive blood smear or no sign of infection in time of sampling. Out of total 52, 11(21.2%) cases infected by *P.vivax*, were HPO compared to the 17(43.6%) cases from total of 39 infected with *P.falsiparum,* The difference is statistically significant (0.03>*P*>0.01>), as is shown in table 3, which suggests higher haemolysis in *P.falciparum*. Excluding HPO showed no significant association between *P.falciparum* and *P.vivax* with three common phenotypes of haptoglobin. Finally, because we could not be sure if three common phenotypes of haptoglobin were same in showing HPO or not, it was not possible to know if the excluding HPO statistical analysis (table 4) was correct or not. We had to resample HPO individuals after treatment and raising their blood HP level and find their phenotypes. Of course, the two other significant associations in table 1 (HP2-2) and table 3(HP2-1) may have been altered by that.

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References

1. Edward T, Ryan (2003). Tropical & geographic medicine Center Division of Infectious Diseases, Massachusetts General Hospital Jackson 504, 55 Fruit Street Boston, Massachusetts 02114 USA, Personal letter.
2. Rougmont A, Bouvier M, Perrin, Yerly S, et al (1988). Hypohaptoglobinemia as an Epidemiological and Clinical Indicator for Malaria, *The Lancet*, 24:709-12
3. Giblett ER, Brooks LE (1963). Haptoglobin Subtypes in Three Racial Groups, *Nature*, 197:576-77
4. Hill AVS, Whithhouse DB, Bowden DK, et al (1987). Ahaptoglobinemia in Melansia: DNA and Malaria Antibody Studies. Transactions of the Royal Society of *Tropical Medicine and Hygien*, 81:573-77.
5. Trape JF, Fribourg- Blanc A (1988). Ahaptoglobinemia in African Population and its Relation to Malaria Endemicity0, *Am J Epidemiol*, 127:1282-87
6. Smithies O (1955). Zone Electrophoresis in Starch Gel: Group Variation in the Serum Proteins of Normal Human Adults *Biochem J*, 61:624
7. Woolf B (1955). An Estimating the Relation between Blood Groups and Diseases *Anu Human Genet*, 19:251-53
8. Yearly S, Bouvier M, Srivastava I, Perin LH (1990). Development of a Haptoglobin ELISA, its Use as an Indicator for Malaria. *Acta Tropical*, 47:237-44.
9. Padma TV, asantha V (1988). *Human Hedrdity*, 38:367-71
10. Trape JF, Fribourg-Blanc A, et al (1985). Malaria Cause of Ahaptoglobinemia in Africans, *Trans Royl Soc Trop Med Hyg*, 79:430-34
11. Farhud DD, Amirshahi P (1985). Ahaptoglobinemia in Favism Patients from Iran, *Acta Anthropogent*, 9(4):206-13