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### Short Communication

## The Rate of *Plasmodium vivax* Infectivity within Glucose-6-Phosphate Dehydrogenase (G6PD) Deficient Individuals in Hormozgan Province, Iran

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### **Abstract**

**Background:** One of the most important enzymatic disorders that interact with malaria is deficiency of G6PD (Glucose-6-phosphate dehydrogenase). This enzyme protects red blood cells from hydrogen peroxide and other oxidative damages. Distribution of this enzyme deficiency usually accompanies with low level distribution of malaria disease in most malarious areas. So this hypothesis may be considered that the G6PD deficiency could be protective against malaria.

**Methods:** Totally 160 samples were taken from *vivax* malaria infected and non-infected individuals. Preparing blood smears and quantitative test for G6PD deficiency were employed for all of the samples. To ensure accuracy of the malaria in negative samples besides using microscopical examination, semi-nested multiplex PCR was also performed for the two groups.

**Results:** In microscopical examination 36 and 124 samples were *vivax* malaria positive and negative respectively. Out of 36 *P.vivax* positive cases 3 (8.3%) cases were detected to be G6PD deficient versus 30 (24.2%) cases out of 124 *P. vivax* negative cases. The results showed a significant differentiation between *P. vivax* positive and *P. vivax* negative cases in the rate of G6PD deficiency (3/36 in positive cases versus 30/124 in negative cases) ( $P < 0.05$ ).

**Conclusion:** *vivax* malaria positive individuals with G6PD deficiency showed too mild symptoms of Malaria or even asymptomatic.

## Introduction

Malaria is the most important and common parasitic disease in the world caused by a protozoan of the genus *Plasmodium*. The Parasite is transmitted to humans via the bite of female *Anopheles* mosquito. Four species including *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae* cause the disease in human (1). More recently *P. knowlesi* is supposed to be the fifth species of human malaria (2). According to the report of World Health Organization (WHO), 216 million new cases with 655000 deaths were reported in 2010. Moreover, transmission of malaria takes place in 99 countries and the disease is reported from 106 countries (3).

Many factors mostly genetic disorders such as thalassemia, sickle cell anemia (HbS), G6PD (glucose-6-phosphate dehydrogenase) deficiency and Duffy blood groups in human can be involved in the process, severity and non-severity of the disease (4). One of the most important disorders involves with malaria is deficiency of G6PD enzyme. Distribution of G6PD deficiency usually accompanies with low level of malaria distribution in the world (5). So this hypothesis may be considered that the G6PD deficiency could be protective against malaria. Deficiency of G6PD enzyme is the most common enzymatic disorders in human with about 400 million cases in the world (5, 6). This disorder is an X-linked inherited phenomenon (4, 5). According to the WHO report, 7.5 percent of the world population has one or two genes for G6PD deficiency and 2.9% suffers from the enzyme deficiency (7).

G6PD enzyme catalyzes the first reaction in pentose phosphate pathway and causes convert G6P (glucose-6-phosphate) to 6-phosphogluconolactone and simultaneously reduces NADP (nicotinamide adenine dinucleotide phosphate) to NADPH. NADPH is the reduced form of NADP and is necessary to protect red blood cells from hydrogen perox-

ide and other oxidative damages (5, 6). There are different varieties of G6PD deficiency in the world. GD A<sup>-</sup> is most distributed variant in Africa and distribution of this defect is very similar to *falciparum* malaria distribution and is thought that *falciparum* malaria causes a natural selection of the maintenance and retention of G6PD deficiency gene in this region (4). In the most Mediterranean countries, including Iran, Mediterranean variety is more frequent than other mutations (6, 7, 9). Although the relationship between G6PD deficiency and *falciparum* malaria has been proven in some studies but relationship between *vivax* malaria and G6PD deficiency needs further studies. Particularly if we know primaquine, a deleterious drug for G6PD deficient individuals, is administered to prevent the relapse in *P. vivax* infection. *Vivax* malaria is in the majority outside of Africa with a global burden approximately 71-391 million clinical cases per year (10). Malaria is an endemic infection in southeast of Iran including Sistan and Baluchistan, Hormozgan and Kerman provinces. Eighty five percent of malaria infection in Iran is reported from the provinces with the majority of *vivax* malaria cases (1). The average incidence of G6PD deficiency is estimated between 10-21% in Iran (6).

This study was proposed to survey the situation of G6PD deficiency within *vivax* malaria infected cases comparing with non-infected individuals in Hormozgan Province, located at the north coast of Persian Gulf in Iran.

## Materials and Methods

A total of 160 *vivax* malaria suspected and non-suspected individuals were enrolled for this study. At the enrollment time clinical sign of fever was measured as probable malaria infected case. From each enrolled individuals a five ml blood for determination of G6PD enzyme deficiency, PCR examination and pre-

paring thin and thick smears were deposited into test tubes. Few drops of collected blood were used to prepare thin and thick smears. Three ml of the blood was poured into EDTA treated test tube and stored at -20 °C for PCR processes, and the rest of the collected blood was deposited into the Acid citrate dextrose - A (ACD-A) predosed test tube for G6PD examination and refrigerated at 4 °C but not more than 21 days (11). Methods of Fluorescent Spot Test (FST) and Dichlorophenol Indophenol (DPIP) can be used to determine situation of G6PD based on the qualitative measurement as described by Farhud and Yazdanpanah (7), but in this study the quantitative measurement method has been employed using Baharafshan kit (Baharafshan Co., Iran).

The semi nested multiplex PCR technique was used for detection of malaria parasites. A rapid DNA extraction method was conducted to extract DNA of the isolated parasites (12), using ROCHE extraction kit. The 18SSrRNA gene with 350-500 bp bearing low repeatability reaction was used to minimize homology of *P. vivax* with other *Plasmodium* species. DNA was amplified using primers 5' - AGTGTG-TATCAATCGAGTTTC - 3' (PLF) as a forward primer and 5' - AGGACTTCCAA-GCCGAAGC - 3' (VIR) as a reverse primer. A reverse primer 5' - AGTTCCCCTAGAA-

TAGTTACA - 3' was employed for *P. falciparum* as a positive control.

## Results

Out of 80 malaria suspected individuals 36 (45%) cases were microscopically detected as *vivax* malaria infection and the rest using both microscopical and semi-nested multiplex PCR methods were diagnosed as non-infected cases. Three (8.3%) cases out of 36 *vivax* malaria infected individuals were found as G6PD deficient cases, while among the 124 non-infected individuals 30 (24.2%) cases were revealed to suffer from G6PD deficiency disorder (Table 1).

Parasite counting showed a range of 200-800 parasitaemia per micro liter of blood. The bio - data obtained from the enrolled individuals indicated that 76.9% and 23.1% were male and female respectively (Table 1). In *vivax* malaria suspected group 36 (45%) cases were positive and the rest 44 (55%) were detected as negative cases using microscopical examination. All control samples remained negative. Thirty nine out of eighty *vivax* malaria suspected individuals were Iranian and the rest were Afghani people. In control group also 66 and 14 individuals were Iranian and Afghani respectively (Table 1). All microscopical negative samples were confirmed by semi - nested multiplex PCR method (Fig. 1).

**Table 1:** *vivax* malaria infection among G6PD deficient and non-deficient individuals including nationality and gender in Hormozgan Province, Southern Iran

Variable		G6PD				Total
		Deficient		Non-deficient		
Nationality		Positive	Negative	Positive	Negative	
Iranian	Male	0	15	3	50	68
	Female	1	7	3	26	37
Afghani	Male	2	8	27	18	55
	Female	0	0	0	0	0
Total		3	30	33	94	160



- positive control (1)
- negative control(2)
- samples (3-9)
- marker 100bp(M)

**Fig. 1:** Semi-nested Multiplex PCR-product of controls and negative samples

## Discussion

This study was conducted in Hormozgan Province a malaria endemic area with dominant infection of *P. vivax*. According to the treatment guideline of *vivax* malaria in Iran primaquine, a member of 8-aminoquinoline group is administrated 0.25 mg/kg as an antirelapse for 14 successive days or 0.75 mg/kg once per week for 8 weeks. Primaquine is listed among the adverse substances for G6PD deficient individuals due to its hemolytic and destructive effect on the red blood cells. Therefore, G6PD deficiency poses an important impediment for primaquine to be used as a powerful antirelapse among the G6PD deficient *vivax* malaria infected patients. On the other hand, detecting the rate of *P. vivax* infection within G6PD deficient cases will help the malaria policy makers for correct managing the treatment and control of the infection. A study shows a rate of 10-21% G6PD deficiency including mostly Mediterranean varieties in Iran (6).

Although relation between G6PD deficiency and *falciparum* malaria in some studies has been

proven (13, 14), few studies have tried to consider the relation between G6PD deficiency and *P. vivax* infection.

In a serological survey conducted by Edrisian and colleagues in 1983 as a pioneer study about relation between G6PD deficiency and malaria infection in Iran they did not find any significant relation between G6PD deficiency and infectivity of *falciparum* malaria in Hormozgan province (15). In another study one Afghani immigrant *falciparum* malaria infected case with G6PD deficient was reported from Iran (14). According to our knowledge this study is the first study conducted in the field of relation between *vivax* malaria and G6PD deficiency in Iran.

The results of this study showed no significant difference between male and female in prevalence of G6PD deficiency with 20.32% and 21.62% respectively in both *P. vivax* infected and non-infected individuals. Prevalence of the deficiency based on the gender more or less is similar to prevalence level reported by Rebholz and colleagues from Tajikistan (16). The results of this study indicated that G6PD deficiency assumes to be a preventive factor against *vivax* malaria infection in the most of studied individuals, similar to those results obtained by Rebholz and colleagues in Tajikistan (16) and Matsuoka et al., from Indonesia (17).

In impact phenotypic and genotypic studies conducted previously by Leslie and colleagues within some Afghani refugees in Pakistan, more or less, as similar as our results had been collected (4). Conversely, Shimizu and colleagues in a molecular based study in the field of G6PD deficiency, Southeast Asian Ovalocytosis (SAO) and *P. vivax* resulted that there was not apparent effect of G6PD deficiency on malaria protection within the studied population, but low parasitaemia was found in the *P. vivax* infected individuals (18). Low parasitaemia density with the malaria infected individuals in our study confirms the results of Shimizu and colleagues results.

## Conclusion

Although G6PD deficiency is assumed to be an *vivax* malaria-resistant genetic trait, more studies are needed to confirm such specialty for more strains of *P. vivax* in the malaria endemic areas.

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