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Original Article

Prevalence of glucose-6-phosphate dehydrogenase (G6PD) deficiency in southeast Iran: implications for malaria elimination

Seyed Mehdi Tabatabaei[1], Alireza Salimi Khorashad[1], Mohammad Sakeni[2], Ahmad Raeisi[3], Zahra Metanat[4]

[1] *Infectious Diseases and Tropical Medicine Research Center, Zahedan University of Medical Sciences, Zahedan, Iran*
[2] *Sistan and Balouchestan Provincial Health Center, Zahedan University of Medical Sciences, Zahedan, Iran*
[3] *School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
[4] *Provincial Clinical Genetic Counseling Center, Zahedan University of Medical Sciences, Zahedan, Iran*

Abstract

Introduction: Glucose-6-phosphate dehydrogenase deficiency (G6PD) is an X-linked genetic disorder with a relatively high frequency in malaria-endemic regions. It is an obstacle to malaria elimination, as primaquine administered in the treatment of malaria can cause hemolysis in G6PD-deficient individuals. This study presents information on the prevalence of G6PD deficiency in Sistan and Balouchetsan province, which hosts more than 90% of *Plasmodium vivax* malaria cases in Iran. This type of information is needed for a successful malaria elimination program.

Methodology: A total of 526 students were randomly recruited through schools located in southeast Iran. Information was collected by interviewing the students using a structured questionnaire. Blood samples taken on filter papers were examined for G6PD deficiency using the fluorescent spot test.

Results: Overall, 72.8% (383/526) of the subjects showed normal G6PD enzyme function. Mild and severe G6PD deficiency was observed in 14.8% (78) and 12.2% (64) of subjects, respectively. A total 193/261 males (73.9%) and 190/265 (72%) females had normal enzyme activity. Mild G6PD deficiency was observed in 10.8% (28) and 18.9% (50) of male and female subjects, respectively. However, in comparison with females, a greater proportion of males showed severe enzyme deficiency (15.3% versus 9.1%). All these differences were statistically significant ($p < 0.006$).

Conclusions: G6PD deficiency is highly prevalent in southeast Iran. G6PD-deficient individuals are susceptible to potentially severe and life-threatening hemolytic reactions after primaquine treatment. In order to achieve malaria elimination goals in the province, G6PD testing needs to be made routinely available within the health system.

Key words: glucosephosphate dehydrogenase deficiency; Iran; malaria; prevalence.

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Introduction

G6PD deficiency is an enzymatic disorder of red blood cells in humans which is inherited in an X-linked recessive pattern [1]; it is the most common genetic defect. An estimated 400 million people worldwide have G6PD deficiency. High frequencies of G6PD-deficient variants have been reported from Africa, Asia, the Mediterranean, and the Middle East [2]. Altogether, 160 mutations, which can result in functional variants presenting with a variety of biochemical and clinical phenotypes, have been described in the G6PD gene [3]. Most G6PD-deficient individuals never experience any signs or symptoms, but some present with episodic hemolytic anemia. The clinical manifestations of G6PD deficiency include a wide range of hemolytic syndromes. G6PD deficiency is most frequently expressed as acute hemolytic anemia and neonatal jaundice, which is usually elicited by external oxidant agents [4]. Exposure to certain medications and chemicals, such as dapsone, methylthioninium chloride (methylene blue), nitrofurantoin, phenazopyridine, primaquine, rasburicase and tolonium chloride (toluidine blue) [5]; ingestion of fava beans [6-8]; topical application of henna [9,10]; and infections [11-13], have been reported to trigger hemolytic anemia in G6PD-deficient individuals. Chronic hemolysis associated with some G6PD variants is likely to result in congenital non-spherocytic hemolytic anemia [14].

The world witnessed a remarkable reduction in the burden of malaria between 2000 and 2010. A large number of countries have achieved elimination (*i.e.*, the absence of transmission in the country) [15] or have been advancing towards malaria elimination [16]. Radical cure of asymptomatic infection and chronic infection by both *Plasmodium falciparum* and *Plasmodium vivax* is the main strategy in reducing transmission of malaria parasites and attaining malaria elimination [17]. A large burden of *P. vivax*, which is more difficult to eliminate than *P. falciparum*,poses a significant barrier to malaria elimination [18]. The latent hepatic stages of *P. vivax* are mainly responsible for subsequent relapses after the first clinical episode [19]. Primaquine is an important medication for case management, whether it is *P. vivax* or *P. falciparum* malaria. It is the only drug that is active against the relapsing forms of *P. vivax* [20] and also has a gametocidal effect on *P. falciparum* [21]. However, one of the major drawbacks in primaquine administration is that it can trigger hemolytic reactions in patients with G6PD deficiency. On the other hand, malaria has been implicated in the spreading of G6PD-deficient variants in malaria-endemic areas [2,22]. It has been hypothesized that G6PD deficiency may incur partial protection against malaria infection [23], and some studies have reported that G6PD deficiency is associated with approximately 50% protection against severe *P. falciparum* malaria [24,25].

Sistan and Balouchetsan is one of the only three provinces in Iran with local malaria transmission. During 2010, a total of 2,380 (72%) of 3,271 malaria cases were diagnosed in this province, and it now hosts more than 90% of the annual burden of malaria cases in the country. Hence, it could be assumed that a significant proportion of people residing in this province have some degrees of enzyme deficiency. Now that the country is in the elimination phase of malaria, a thorough understanding of the prevalence of G6PD deficiency could be of great value in a region in which malaria is still a public health problem. This study aims to present updated information on the prevalence of the distribution of individuals at risk of primaquine-induced hemolysis in Sistan and Balouchetsan province, which is required in the planning and implementation of the malaria elimination program.

Methodology

A cross-sectional study was conducted between September and December 2010 in Sistan and Balouchestan province, southeast Iran. The study population comprised healthy male and female students attending schools in urban and rural areas. Based on the data from previous epidemiological studies [26], given that prevalence of G6PD deficiency was estimated to be approximately 20% ($\alpha = 0.05$, $\beta = 0.2$, and $d = 0.04$), and after taking into account the effect of the study design, the estimated sample size was calculated as 516 students across all schools. Multi-stage cluster sampling was used to recruit subjects. The schools were randomly selected from a list of schools for each district. The number of students approached from each school was determined using probability proportional to size based on the population of the students during the school year 2009. The sample included an equal number of boys and girls.

A 20-item structured questionnaire was developed for eliciting information. The questionnaire included three categories of questions: a) demographic characteristics such as age, sex, residence, and whether parents were first cousins; b) history of hemolytic reactions after exposure to oxidant agents, including history of neonatal jaundice and blood exchange transfusion for management of jaundice, hemolytic reactions following ingestion of fava beans, local application of henna, or intake of anti-malaria medicines, all based on what the subjects recalled from their medical records; and c) history of malaria in the participant and the participant's household members.

The participants were interviewed by trained health workers during school visits. After the questionnaire was completed, blood samples were taken using filter paper. All filter paper samples were coded and sent to the reference laboratory on the same day samples were taken. All tests were performed by a trained laboratory technician. A Kimia Pajouhan test kit (Iran Medical Equipment and Medical Engineering Company, Tehran, Iran), which uses the Beutler fluorescent spot method, was used to detect G6PD deficiency [27]. The semi-quantitative fluorescent spot test described by Beutler [28] and subsequently modified [29], was used to identify enzyme defects such as galactosemia and G6PD deficiency. This is a rapid, simple, sensitive, and inexpensive test that visually detects NADPH produced by G6PD under ultraviolet light [30,31]. The fluorescent spot test is reliable for the detection of hemizygous males and homozygous females with a sensitivity and specificity of 100% and 99%, respectively [31]. However, it is only moderately reliable for detection of G6PD deficiency in heterozygous females [32,33]. According

to the instruction from the Kimia Pajouhan test kit brochure, samples with more than 80% G6PD function (strong fluorescence) were considered as normal, those with an enzyme activity of 40%–80% (moderate fluorescence) as mild deficiency, and subjects that showed less than 40% G6PD function (no fluorescence) were classified as severe deficiency variants.

Continuous variables were tested for normality of distribution using Kolmogorov-Smirnov goodness of fit tests. Categorical variables were presented as counts and percentages. The Chi-square test and Fisher's exact test were used to compare the distribution of categorical variable between different groups. A p value < 0.05 was considered significant for all analyses. Data analysis was performed using SPSS version 20 statistical software package.

Ethical approval for this study was obtained from Zahedan University of Medical Sciences Ethics Committee. Oral consent was obtained from participants' parent(s) and from the students by school authorities before individual surveys were conducted.

Results

A total of 526 subjects participated in the study (261 males and 265 females). The mean age was 14.1 ± 1.2 years (range, 10–20 years). The demographic characteristics of the participants are presented in Table 1. Given the design of the study and sampling method, the distribution of subjects based on gender, age group, and site of residence was relatively similar. Parents were first cousins in 111 male (42.5%) and 120 female (45.3%) subjects.

Table 2 shows the participants' history of hemolytic reactions and jaundice following exposure to oxidant agents. Only 10 male (3.8%) and 18 female participants (6.8%) reported a history of hospital admission due to neonatal jaundice. Of those cases hospitalized, 2 males (0.8%) and 5 females (1.9%) mentioned receiving exchange blood transfusion for management of neonatal jaundice. Hemolytic reaction following consumption of fava beans (favism) was reported in 1.5% (4 cases) and 4.9% (15 cases) of male and female participants, respectively. Only 3 female respondents (1.1%) mentioned that they had experienced hemolytic reactions as a consequence of local application of henna. Jaundice and hematuria after taking anti-malarials (mostly chloroquine and quinine) was reported by 1 male (0.4%) and 2 female (0.8%) participants, respectively. Overall, 10 male students (3.8%) and 13 female students (4.9%) had a history of contracting malaria. History of malaria infection in family members during the past 12 months was present in 7 male (2.7%) and 13 female (4.9%) subjects.

In general, 72.8% of the subjects investigated showed normal levels of G6PD enzyme activity. Mild and severe G6PD deficiency was observed in 14.8% and 12.2% of subjects, respectively. A total of 193 male (73.9%) and 190 female participants (72%) had normal enzyme activity. Mild G6PD deficiency was observed in 10.8% and 18.9% of male and female subjects, respectively. However, in comparison with females, a greater proportion of males were found to have severe deficiency (15.3% versus 9.1%), and the difference observed was statistically significant (p

Table 1. Socio-demographic characteristics of participants by gender, 2010 (n = 526)

Variables		Male n = 261 (49.6%)	Female n = 265 (50.4%)	$\chi 2$ p value
Age group (years)	≤ 13	64 (24.4)	61 (23.0)	0.058
	14–15	163 (62.5)	185 (69.8)	
	≥ 16	34 (13.0)	19 (7.2)	
Residence	Urban	119 (45.6)	123 (46.4)	0.850
	Rural	142 (54.4)	142 (53.6)	
District	Zahedan	69 (26.4)	70 (26.4)	0.999
	Khash	21 (8.0)	20 (7.5)	
	Iranshahr	50 (19.2)	55 (20.8)	
	Saravan	41 (15.7)	42 (15.8)	
	Sarbaz	16 (6.15)	16 (6.0)	
	Nikshahr	21 (8.0)	19 (7.2)	
	Konarak	10 (3.8)	11 (4.2)	
	Chabahar	33 (12.6)	32 (12.1)	
Parents are first cousins	Yes	111 (42.5)	120 (54.3)	0.779
	No	140 (53.6)	134 (50.6)	
	Unknown	10 (3.8)	11 (4.2)	

<0.006).

The subjects residing in Chabahar district, located on the coastal areas of the Oman Sea with a significant burden of malaria in the province, showed the highest proportion of G6PD deficiency (37%) (Figure 1), while the prevalence of G6PD-deficient variants in Konarak, Iranshahr, and Khash districts was less than 25%. The proportion of G6PD deficient subjects in Nikshahr, Sarbaz, and Saravan districts ranged from 30% to 32%. The largest proportion of subjects with mild G6PD deficiency was observed in Sarbaz district (25%), while no cases with mild enzyme deficiency were detected in the subjects investigated in Konarak district. The greatest percentage of subjects with severe deficiency was reported from Chabahar (17%), while subjects from Sarbaz showed the lowest proportion of severe G6PD deficiency (6%).

When comparing G6PD status by parents' inbreeding (Table 3) in each gender group, the proportion of male subjects with severe enzyme deficiency was the same (*i.e.*, approximately 15%), regardless of parents' consanguinity status. However, female subjects whose parents were first cousins were more likely to have severe G6PD deficiency (14.2% versus 4.5%). On the other hand, a significantly greater proportion of females whose parents were not relatives showed mild enzyme deficiency (23.3% versus 15%), and the difference was statistically significant ($p < 0.040$).

Table 4 compares parents' consanguinity status, history of hemolytic reactions following exposure to

Figure 1. Proportion (%) of G6PD deficiency in study participants by district, 2010 (n = 526)

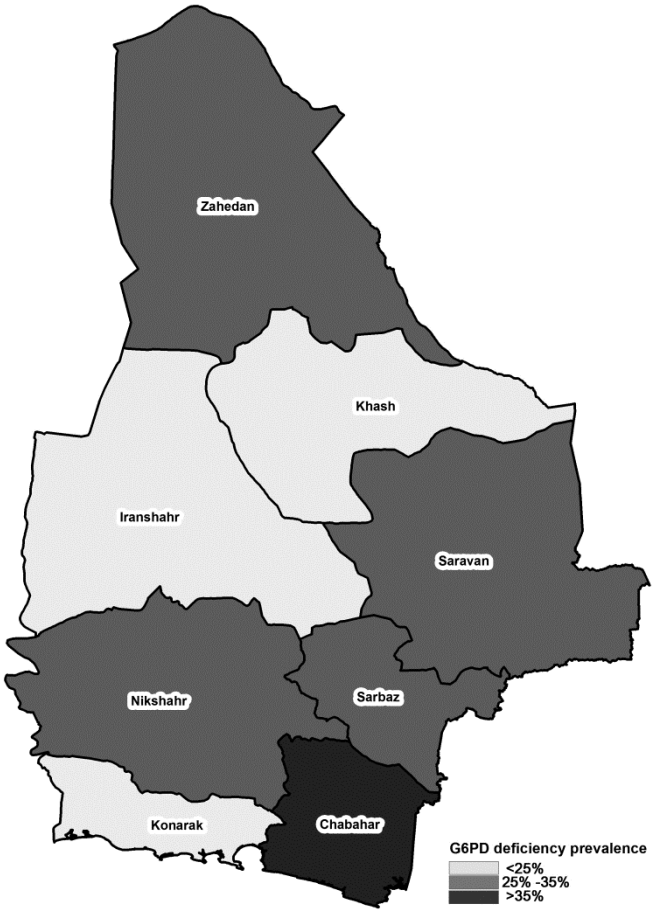

Table 2. Participants' history of hemolytic reactions following exposure to oxidants by gender, 2010 (n = 526)

Variables		Male n = 261 (49.6%)	Female n = 265 (50.4%)	$\chi 2$ p value
History of hospital admission due to neonatal jaundice	Yes	10 (3.8)	18 (6.8)	0.001
	No	196 (75.1)	222 (83.8)	
	Do not recall	55 (21.1)	25 (9.4)	
History of blood exchange transfusion due to neonatal jaundice	Yes	2 (0.8)	5 (1.9)	0.017[1]
	No	214 (82.0)	235 (88.7)	
	Do not recall	45 (17.2)	25 (9.4)	
History of favism	Yes	4 (1.5)	13 (4.9)	0.069
	No	245 (93.9)	237 (89.4)	
	Do not recall	12 (4.6)	15 (5.7)	
History of jaundice and/or hemolytic reactions following local application of henna	Yes	0 (0.0)	3 (1.1)	0.162[1]
	No	254 (97.2)	258 (97.4)	
	Do not recall	7 (2.7)	4 (1.5)	
History of allergic reactions following intake of malaria medications	Yes	1 (0.4)	2 (0.8)	0.846[1]
	No	202 (77.4)	201 (75.8)	
	Do not recall	58 (22.2)	62 (23.4)	
History of ever having contracted malaria	Yes	10 (3.8)	13 (4.9)	0.600[1]
	No	250 (95.8)	252 (95.1)	
	Do not recall	1 (0.4)	0 (0.0)	

[1]P values for Fisher's exact test

oxidant agents and medication, and history of malaria between participants who had normal enzyme activity and G6PD-deficient subjects. Although only three subjects with normal and deficient enzyme activity recalled a history of allergic reactions following intake of anti-malaria medications, those with a normal G6PD variant were more likely to report no hemolytic reactions following exposure to these drugs (79.9% versus 67.6%), and the difference was statistically significant ($p < 0.007$) A greater proportion of normal study subjects reported a history of contracting malaria (5.5%) compared to subjects with impaired G6PD enzyme activity (1.4%), but the difference was not statistically significant. No statistically significant differences were observed in the proportion of consanguinity rate in normal subjects (44.4%) compared with G6PD-deficient participants (43%). Similarly, no differences were observed between participants with normal enzyme activity and those with G6PD deficiency in terms of history of neonatal jaundice, blood exchange transfusion, favism, and hemolytic reactions following application of henna.

Discussion

The results from this cross-sectional study showed that mild and severe G6PD deficiency was present in 14.8% and 12.2% of the study subjects, respectively. Mild G6PD deficiency was more common in females (18.9% versus 10.8%), while a greater proportion of males showed severe enzyme deficiency (15.3% versus 9.1%).

Early detection and management of G6PD

Table 3. Proportion (%) of G6PD enzyme activity levels in study participants by gender and parental consanguinity status, 2010 (n = 526)

Gender	Parents are first cousins	Normal N (%)	Mild deficiency N (%)	Severe deficiency N (%)	$\chi 2$ p value
Male	Yes	85 (76.6)	9 (8.1)	17 (15.3)	0.779
	No	100 (71.4)	18 (12.9)	22 (15.7)	
	Unknown	8 (80.0)	1 (10.0)	1 (10.0)	
Female	Yes	85 (70.8)	18 (15.0)	17(14.2)	0.040[1]
	No	96 (72.2)	31 (23.3)	6 (4.5)	
	Unknown	9 (81.1)	1 (9.4)	1 (9.4)	

[1]P values for Fisher's exact test

Table 4. Participants' parents' consanguinity status, history of hemolytic reactions following exposure to oxidant agents and drugs, and history of malaria by G6PD status, 2010 (n = 526)

Variables		Normal (n = 383) N (%)	Deficient (n = 143) N (%)	$\chi 2$ p value
Parents are first cousins	Yes	170 (44.4)	61 (43.0)	0.633
	No	196 (51.2)	77 (73.8)	
	Do not recall	17 (4.4)	4 (2.8)	
History of hospital admission due to neonatal jaundice	Yes	22 (5.7)	6 (4.2)	0.690
	No	301 (78.6)	116 (81.7)	
	Do not recall	60 (15.7)	20 (14.1)	
History of blood exchange transfusion due to neonatal jaundice	Yes	6 (1.6)	1 (0.7)	0.735[1]
	No	324 (84.6)	124 (87.3)	
	Do not recall	53 (13.8)	17 (12.0)	
History of favism	Yes	8 (2.0)	9 (4.6)	0.055[1]
	No	356 (93.0)	125 (88.0)	
	Do not recall	19 (5.0)	8 (7.4)	
History of jaundice and/or hemolytic reactions following local application of Henna	Yes	2 (2.2)	1 (0.8)	0.111[1]
	No	376 (98.2)	135 (95.1)	
	Do not recall	5 (1.3)	6 (4.2)	
History of allergic reactions following uptake of malaria medications	Yes	2 (0.5)	1 (0.8)	0.007[1]
	No	306 (79.9)	96 (67.6)	
	Do not recall	75 (19.6)	45 (31.6)	
History of ever contracted malaria	Yes	21 (5.5)	2 (1.4)	0.063[1]
	No	361 (94.2)	140 (98.6)	
	Do not recall	1 (0.3)	0 (0.0)	

[1]P values for Fisher's exact test

deficiency at the time of malaria diagnosis is a crucial aspect in the current phases of malaria elimination in Sistan and Balouchestan province, in which more than 90% of malaria cases are due to *P. vivax*. *P. vivax* is able to form latent hypnozoites in infected individuals, which cause relapse episodes following the initial infection. Therefore, the dormant liver stage of *P. vivax* is responsible for a large proportion of *P. vivax* malaria cases and is the principal disease reservoir. In a review of more than 300 studies publishedbetween 1920 and 2010 regarding *P. vivax* relapse rates in patients with and without radical treatment, a total of ~17,000 (19.5%) relapses were reported in over 87,000 patients with acute *P. vivax* malaria, and the relapse rate ranged from zero to 100% [34]. Primaquine is the only licensed medicine for the treatment of *P. vivax* hypnozoites [20]. Moreover, the transmissible stages of *P. falciparum* (*i.e.*, gametocytes) are sensitive to primaquine [35]. As recommended by the World Health Organization (WHO), administering a single dose or short course of primaquine in conjunction with the primary treatment for *P. falciparum* infection can reduce the transmission of the parasite from the patient to mosquitoes [36]. Hence, we will require a wider use of primaquine for both achieving the radical treatment of *P. vivax* and also reducing *P. falciparum* transmission. Although the relapse rate among *P. vivax*malaria cases in Iran has been shown to be less than 28% [37], having a clear view on the status of G6PD deficiency is essential. Primaquine administered to malaria patients with severe G6PD deficiency can cause life-threatening complications such as acute intravascular hemolysis and acute renal failure, which in turn may undermine confidence in this anti-malarial drug [38]. On the other hand, patients with unconfirmed and/or undiagnosed G6PD deficiency who do not receive primaquine for treatment of malaria are highly likely to reduce the impact of this drug [39]. It is imperative to understand the situation clearly, as the country has been aiming to eliminate malaria, and even one case of treatment failure/loss due to unknown G6PD deficiency status is of high importance from an epidemiological point of view.

The prevalence of G6PD deficiency in the Islamic Republic of Iran has been studied in different settings and study populations, mainly newborns and blood donors. The prevalence rates reported in the studies reviewed were not comparable with the prevalence rate observed in Sistan and Balouchestan province. For example, screening of newborns in a Tehran hospital for G6PD deficiency showed that 2.1% (3.6% of males and 0.6% of females) were G6PD-deficient [40]. Similarly, the proportion of the neonates born in the city of Arak hospitals with enzyme deficiency was 2.2% [41]. A study of male and female neonates in the city of Mashhad reported a relatively low prevalence of G6PD deficiency of 0.8% in the total study population with a confidence interval of 0.5% to 1% [42]. G6PD deficiency has been reported in 5.7% and 4.2% of newborn boys and girls, respectively, in the city of Rafsanjan [43]. However, higher proportions of G6PD deficiency (8.4%) have been observed in newborns in the city of Bushehr, a region that used to have a high prevalence of malaria decades ago [44].

The prevalence rates of G6PD deficiency in the male population of Sistan and Balouchestan province were much higher than those observed in similar studies carried out in other provinces. A study from Kermanshah showed that 5.3% of male students were severely G6PD deficient [45]; a frequency of 7.6% was reported from Khuzestan province [46], and it was observed that only 6% of male blood donors in Shiraz were G6PD deficient [47].

G6PD deficiency is an X-linked genetic disorder. Females can thus be homozygously or heterozygously deficient. As compared with the results for G6PD deficiency in women from studies carried out in other provinces, it is evident that the prevalence of G6PD deficiency in female individuals living in this province is much higher than in the rest of the country. The high proportion of G6PD-deficient variants in the female population is highly likely due to preferential expression of the G6PD-deficient gene and X-inactivation of the normal gene. It also could be explained by the presence of an enhancer gene that increases the chance of the expression of the G6PD deficiency [48]. Moreover, consanguinity is a deeply rooted social trend among the Sistan and Balouchestan population. High levels of consanguinity due to increased frequency of first-cousin marriages was observed in the parents of 42.5% and 45.3% of male and female participants, respectively. Theoretically, this could result in a high allele frequency and a relatively high proportion of homozygotes and, consequently, a greater proportion of females with the severe deficiency [49,50]. In our study, this was further substantiated, as we found that female participants whose parents were relatives were more likely to show severe G6PD deficiency. Compared with males whose parents were related, females whose parents were related were more likely to have mild G6PD deficiency. Moreover, a greater proportion of females whose parents did not have a consanguineous

marriage showed mild enzyme deficiency. This could be partly explained by a phenomenon called lyonization, a random inactivation of one of the two X chromosomes [31] that can yield in a mixed (normal and G6PD-deficient) population of erythrocytes in heterozygously-deficient women.

Sistan and Balouchestan province is home to an ethnically diverse population including Balouch, Sistani, and Fars and, to a lesser extent, people from other ethnic backgrounds. Although the majority of the population in the province is comprised of the Balouch ethnic group, mostly residing in the central and southern regions of the province, there is considerable diversity within this group, as they are originally from different tribes and not a common ancestor. This diversity would result in a wide range of micro-geographic differences in the distribution of the frequency of genetic traits, including G6PD deficiency. This was also evident from the results of our study. The proportion of G6PD-deficient subjects widely ranged from 37% in Chabahar to 14% in Konarak and Iranshahr, and this figure changed between 30% and 32% in the remaining districts in the province. Study subjects from Chabahar district were more likely to show severe enzyme deficiency, while the greatest proportion of mild G6PD deficiency was observed in Sarbaz district. The findings from this study are in agreement with the results from a study that reported a significantly higher prevalence of G6PD deficiency in Balouch schoolboys (9.3%) residing in areas with highly endemicity of malaria as compared with 5.8% in Sistani schoolboys [51]. In this study, the highest proportion of G6PD deficiency in Iranshahr (24.5%) and Zahedan showed the lowest prevalence of 6.4%. Another study showed that only 5.9% of male applicants attending pre-marriage counseling and testing in the city of Zahedan were G6PD deficient, which is much lower than those proportions observed in the southern and central districts in the province [52].

Only a small number of study subjects reported an allergic reaction after intake of drugs used for malaria treatment. The results from this study showed that normal G6PD variants are less likely to experience hemolytic reactions following uptake of anti-malaria medications, which is in agreement with studies that have reported that G6PD deficiency is a proven risk factor for allergic reactions following exposure to reactive oxygen species such as anti-malarials [53]. However, 30% and 20% of G6PD-deficient and normal subjects, respectively, did not recall any hemolytic reactions following intake of anti-malaria medications. Hence, recall bias could be an issue when interpreting the results of history of severe allergic reactions in study subjects. Although the difference was not statistically significant, a smaller proportion of G6PD-deficient people had a history of contracting malaria (1.4% versus 5.5%), which is in agreement with the theory that decreased G6PD enzyme activity is associated with some degree of protection against malaria [24].

The fluorescent spot test, the spectrophotometric assay, and the cytochemical assay are used to identify G6PD deficiency [31]. It has been suggested that the gold standard for the diagnosis of the G6PD status is estimating the G6PD enzyme activity, which is the most direct, accessible, and reliable assessment method [17]. The fluorescent spot test used in this study was inexpensive and easy to perform, but only moderately reliable for detection of G6PD deficiency in heterozygous women. The healthy erythrocytes in heterozygous females are able to generate enough NADPH, which in turn can produce fluorescent spots. As a result, the heterozygous deficiency is likely to be overlooked [31]. Therefore, underestimation of the G6PD-deficient variants in the female population is one of the potential limitations of this study.

Another limitation of this study is that a questionnaire was used to collect the participants' medical histories and their past experiences related to the G6PD deficiency status. Therefore, this information should be interpreted with caution, as recall bias could not be ruled out.

Conclusions

Routine administration of primaquine in all *P. vivax* malaria patients, which has been recommended in the National Malaria Management Guidelines and also by the WHO, would be a valuable elimination tool if properly applied. However this strategy requires introduction of 6PD testing in the health system like the one used in this study, or a rapid test at the point of care. If successfully employed, routine G6PD testing could result in significant reductions in reservoirs of *P. vivax* malaria and help towards the goal of malaria elimination in the province.

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Corresponding author

Seyed Mehdi Tabatabaei
Assistant Professor of Epidemiology
Infectious Diseases and Tropical Medicine Research Center
Zahedan University of Medical Sciences
Mashahir Square, Zahedan, Iran
Phone: + 985412438801
Fax: + 985412438800
Email: zu.healthdeputy@gmail.com

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