ORIGINAL ARTICLE

Newborn screening for glucose-6-phosphate dehydrogenase deficiency in Isfahan, Iran: a quantitative assay

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Objectives To determine the prevalence of glucose-6-phosphate dehydrogenase (G6PD) deficiency in Isfahan, the central state of Iran.

Methods From February to March 2006, a total of 2501 samples were screened for the quantitative measurement of G6PD activity by enzymatic colorimetric assay by a commercial kit (GAMMA, Belgium). The neonates were referred from 17 delivery units to the Isfahan neonatal screening center at 3–7 days after birth. Any neonate with a value < 6.4 U/gHb was considered G6PD deficient.

Results Of the 2501 newborns (1307 males, 1194 females) screened, 79 neonates were found to have G6PD deficiency (67 males, 12 females). The overall incidence of G6PD deficiency was 3.2%. Frequency in male population was 5.1 % (67 out of 1307 male neonates) and in female population was 1% (12 out of 1194 female neonates).The female:male ratio was 1:5.5 ($P = 0.0001$). The mean enzyme activity in deficient patients was 3.22 $\pm$ 1.8 U/gHb (male deficient group; 3.17 $\pm$ 1.74 U/gHb, female deficient group; 3.49 $\pm$ 2.17 U/gHb, $P = 0.58$).

Conclusion Routine neonatal screening in Isfahan, Iran with a relatively high prevalence of G6PD deficiency is justified and meets the World Health Organization recommendation.

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INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human enzyme deficiency in the world; it affects an estimated 400 million people.[1] G6PD deficiency is an X-linked disorder affecting mostly African, Mediterranean and far-eastern populations.[2,3] G6PD is the first enzyme of the pentose phosphate pathway and catalyzes the conversion of glucose-6-phosphate to 6-phosphogluconolactone, with the concomitant reduction of nicotinamide adenine dinucleotide phosphate (NADP) to its reduced form (NADPH). NADPH protects cells from oxidative stress. Haemolytic anaemia and neonatal jaundice are the two major pathologies associated with G6PD deficiency. Haemolytic anaemia results from stress factors such as certain oxidative drugs, infections or fava beans.[4] G6PD deficiency is also known as 'fauvism', a term used to describe acute haemolytic anaemia after the consumption broad beans (Vicia faba)[5] – a common food in Iran. During the neonatal period, the disease may manifest as neonatal jaundice, which may cause severe neurological complications and even death in some populations.[6,7] Although the mechanism of jaundice in G6PD deficient neonates is not completely defined, haemolysis dose not seem to contribute as much as impaired bilirubin conjugation and clearance by liver.[8] There is a remarkable association between neonatal jaundice, G6PD deficiency and Gilbert syndrome. The newborns that are homozygous for both abnormalities are particularly at risk for neonatal jaundice with pathogenesis of decreased bilirubin conjugation and elimination.[9,10]

Neonatal screening for G6PD deficiency has long been established in many countries with high disease prevalence. The World Health Organization recommends screening all newborns in populations with a prevalence of 3–5% or more in males.[11] Newborn screening for G6PD deficiency is not performed routinely in the Iran, because there are limited and no reliable data about prevalence of this disease in Iran.

The aim of this study was to determine the prevalence of G6PD deficiency in a quantitative assay in Isfahan, the central state of Iran.

MATERIALS AND METHODS

The enrolled neonates were delivered at Isfahan, the central state of Iran, during the first three months of 2007. All neonates born in 17 maternity hospitals of Isfahan were referred for screening. A 2 mL venous blood sample was collected on the day of referral (3rd–7th day of birth) from each neonate by trained nurses, from the cubital vein, in ethylene diamine tetra acetic acid (EDTA) tubes and delivered immediately to the laboratory where investigations were carried out within 24 hours. The red cell G6PD activity, expressed as units per gram of haemoglobin (U/gHb), was determined by an enzymatic colorimetric assay for the quantitative determination of G6PD deficiency using a commercial kit (GAMMA, Belgium). The assay was performed according to the instructions included in the kit. On the basis of frequency

distribution of activity levels, the critical level for diagnosing G6PD deficiency was considered 6.4 U/gHb.[12,13] Any neonate with an activity below this value was diagnosed as G6PD deficient.

The Ethics Committee of Isfahan University of Medical Sciences approved the study, and informed written consent was obtained from mothers before delivery.

The neonates' sex, weight, height, head circumferences, gestational age, maternal age and parents' consanguinity were recorded on questionnaires and transferred to coding sheets in a computer database. Data analyses were performed using SPSS version 10.5 (Chicago Inc., USA). Frequency, mean and SD for demographic data, G6PD activity levels in neonates are presented. Mean values were compared by the student's *t* test. Gender and consanguinity differences between groups were analysed by chi-square test. Statistical significance was considered to be at the 5% probability level.

RESULTS

Of 2560 subjects enrolled in the study, 59 (37 males and 22 females) were excluded, either because of clotted or insufficient blood samples, or because the parents did not give consent for sampling. Therefore, of the 2501 newborns (1307 [52.3%] males, 1194 [47.7%] females) screened over a period of nearly three months, 79 neonates were found to have G6PD deficient (67 males, 12 females). The overall incidence of G6PD deficiency was 3.2%. Frequency in male population was 5.1% (67 neonates of 1307 male neonates) and in female population was 1% (12 neonates of 1194 female neonates). The female:male ratio for G6PD deficiency was 1:5.5 (Table 1).

There was no difference in the prevalence of parental consanguinity among female G6PD deficient and female normal infants. Also, no statistical gestational age difference was found between the two groups (Table 1).

The mean $\pm$ SD enzyme activity in deficient patients ($n = 79$) and normal G6PD patients ($n = 2422$) were 3.22 ± 1.8 U/gHb and 35.12 ± 12 U/gHb, respectively. There was a statistically significant difference between the mean of enzyme activity in deficient patients and normal G6PD patients ($P < 0.0001$).

There was gender difference in enzyme activity when the data of all newborn infants (G6PD deficient and normal infants) were analysed together (34.99 ± 12.32 versus 33.32 ± 13.67 U/gHb; $P = 0.001$ in female and male infants, respectively). No significant differences in G6PD deficiency were noted between term and premature neonates (Table 1).

Table 2 gives the activity of G6PD among neonates studied in both groups according sex. Although the incidence of G6PD deficiency is significantly higher in males (Table 1) but as shown in Table 2, the mean G6PD activity were not significant different between males and females in G6PD deficient subjects and in G6PD normal group.

Table 2 Activity of glucose-6-phosphate dehydrogenase (G6PD) among neonates studied in both groups according sex

	G6PD activity, U/g Hb			
	Range	Mean	SD	*P* value
G-6-PD deficient				
Male	0.6–6.3	3.17	1.74	0.58
Female	0.4–6.2	3.49	2.17	
G-6PD normal				
Male	6.9–67	34.94	12.04	0.45
Female	6.9–65	35.31	11.96	

SD, standard deviation; NS, non-significant

DISCUSSION

Our data suggests the prevalence of G6PD is relatively high in the Isfahan central area of Iran (3.2% in general population and 5.1% and 1% in male and female population, respectively). There has been no precise previous information about the incidence of G6PD deficiency in neonates of this part of Iran. Several studies have reported incidence in other parts of Iran, and the prevalence varies markedly among different provinces. These studies gave a high rate of 8.65%–16.4% in the northern part (Mazandaran and Guilan Provinces), 12% in the southern part (Shiraz) and 19.3% in the southeastern part of Iran.[14] One study from cord blood screening of newborns for G6PD deficiency in 2000 neonates in two hospitals in Tehran (the capital of Iran which lies between the centre and the north) revealed a relatively lower rate (2.1% of total population, 3.6% males) compared with other reports.[14] These differences may be due to different genetic types of G6PD in different caste and ethnic groups in Iran. Iran is a large country of approximately 70 million people, with different ethnicity in northern, southern and central provinces. In most areas of high prevalence of G6PD deficiency, several G6PD mutations are found.[8] The first most common variant in the coastal provinces of Caspian Sea in the north of Iran and in the Kurdish population of Western Iran is G6PD Mediterranean.[15,16] Our method in this study was only a phenotypic test and there are not any exact data about common variants in center of Iran, Isfahan. More research is needed to evaluate the prevalence of G6PD deficiency and the association of G6PD variants with the population of all parts of Iran. In addition, overall enzymatic activity and G6PD deficiency incidence depends on different laboratory methods and kind of blood specimens (on filter paper or in heparinized tubes).[13] Various screening methods have

Table 1 Demographic details of neonates studied

Parameter		G-6-PD deficient	G-6-PD normal	Significance
N (%)		79 (3.2%)	2422 (96.8%)	0.0001
Sex	Male	67 (5.1%)	1240 (94.9%)	0.0001
	Female	12 (1%)	1182 (99%)	
Maturity	≥38 week	68 (2.9%)	2231 (97.1%)	NS
	<38 week	11 (5.4%)	191 (94.6%)	
Parents marriage in female cases	Relative	3 (25%)	300 (75%)	NS
	Not relative	9 (25.4%)	882 (74.6%)	

G-6-PD, glucose-6-phosphate dehydrogenase; NS, non-significant

been reported, including qualitative enzyme activity detection by the fluorescent-spot test and semiquantitative or fully quantitative methods.[17] The qualitative or semiquantitative methods might be not sufficient to detect all heterozygote females.[12,17,18] One study from Greece reported that a high percentage of partially deficient female neonates are missed during routine semiquantitative method, which uses a cut off of 2.1 U/gHb. This study proposed a fully quantitative G6PD screening kit and suggested that any neonate with an activity below 6.4 U/gHb should be considered as G6PD deficient.[12] In the present study, the first report of G6PD deficiency among the all neonates born in Isfahan was made using a fully quantitative method and a cut off of 6.4 U/gHb. In our study, the male to female ratio in G6PD deficient neonates was 5.5:1. In other studies in the city of Dhahran (Saudia Arabia),[19] Yanbu(Saudi Arabia),[20] Pinjab (India),[21] Tehran (Iran)[14] male to female ratio in G6PD deficient neonates was reported as 2:1, 3:1, 1.8:1 and 6:1, respectively. It seems that the incidence of G6PD deficiency in the Iranian female population is lower than in other female populations. This unexplained finding is not related to screening method because we used a fully quantitative G6PD screening kit with a high cut-off point.

In conclusion, it is necessary to establish the frequency of G6PD deficiency among the different ethnic groups living in Iran, because The World Health Organization recommends screening all newborns in populations with a prevalence of 3–5% or more in males.[11] Routine neonatal screening in Isfahan, where there is a relatively high prevalence of G6PD deficiency, is logical.

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