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SCREENING

Neonatal screening for glucose-6-phosphate dehydrogenase deficiency fails to detect heterozygote females

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Abstract. We examined glucose-6-phosphate dehydrogenase (G6PD) deficiency in north-eastern Italian Caucasian neonates detected by neonatal screening, in order to measure the incidence of heterozygote females detected by neonatal screening, and to estimate the near-true total incidence. A total of 85,437 Caucasian neonates, born between January 2000 and December 2001, have been enclosed in the study. The total incidence of the disease, measured by fluorescent method, is 0.9‰; the total incidence, calculated by Hardy–Weinberg law, is 4.8‰. The frequency of missed females is 93% of total females expected with G6PD deficiency; most of them are very likely heterozygous females. The sensitivity of the fluorescent method might be not sufficient to detect all females. Since heterozygote females might develop the symptoms of G6PD deficiency later, these results suggest that the G6PD neonatal screening may not be helpful in preventing disease in females.

Key words: Glucose-6-phosphate dehydrogenase (G6PD), Newborn screening

Abbreviation: G6PD = glucose-6-phosphate dehydrogenase

Introduction

Glucose-6-phosphate dehydrogenase deficiency (G6PD; OMIM 305,900) is an *X*-linked disorder affecting red-cell metabolism. It is one of the most common genetic diseases in the world, and its symptoms (haemolytic anaemia, jaundice, and kernicterus) [1] may be avoided with a prompt diagnosis. For this purpose, a neonatal screening for G6PD deficiency was established in many countries [2].

The distribution of G6PD deficiency in different populations is highly correlated with current or past malaria endemicity [3]. It is commonly described in North Africa, Spain, Portugal [4] and Greece [5]. In Sardinia, its distribution is well known [6, 7], however in continental Italy it is poorly investigated [8]. In Veneto region, located in northeast Italy, the overall incidence, measured during 16 years of neonatal screening, is 2.4‰ [9].

Because G6PD gene (Gd) is *X*-linked, in males it occurs only as hemizygote normal (Gd+) or deficient (Gd−) status; in females it occur as normal homozygotes (Gd+/Gd+), deficient homozygote (Gd−/Gd−) or heterozygote (Gd−/Gd+) status: the last phenotype is often defined as 'intermediate'. Although deficient heterozygote females may show a wide range phenotype due to the variable *X* inactivation process during embryogenesis, they are at increased risk of haemolysis (relative risk 2.26) as well as deficient homozygote (relative risk 2.68) [10] and may develop the disease also if the neonatal screening is negative [11]. Even though, semi-quantitative tests do not miss hemizygote deficient males, they fail to detect most of the heterozygote females [12]. We examined G6PD deficiency in north-eastern Italian neonates detected by fluorescent method, in order to measure the incidence of heterozygote females detected by neonatal screening, and to estimate the near-true total incidence.

Subjects and methods

A total of 85,437 Caucasian neonates, born between January 2000 and December 2001 in Veneto region, were included in the study. Screening results (positive or negative) and quantitative G6PD activity in erythrocytes (in positive samples only) were reported.

The screening protocol was as follows: a heel capillary blood sample was taken from each newborn on the 3rd–5th day of life, adsorbed on filter paper (Schleicher and Schuell, Inc., Keene, NH 03431) and mailed to our neonatal screening laboratory, where fluorescent spot-test was applied to detect G6PD deficiency. The spot-test is essentially the same of the classic Beutler method [5]. The results were classified

as fluorescent negative (−) or positive including both bright (++) and weak (+) positive. Neonates with positive test were submitted to quantitative analysis with standard procedure [13]. G6PD activity was expressed as international units per gram of haemoglobin (U/g Hb). A mean of 12 ± 2 U/g Hb was considered as normal reference value. A neonate with residual enzyme activity lower than 60% of normal activity was classified as 'G6PD deficient' [12].

In a randomly mating population, G6PD gene frequency is suggested by affected males' frequency. According to Hardy–Weinberg low [14, 15], p and q are the frequencies of the normal and deficient allele, respectively. We applied Hardy–Weinberg equation to our populations, subdivided by sex, to know measured and expected incidence of G6PD deficiency. Statistical analyses were performed with SPSS v.11.0 software for Windows package for personal computers (SPSS, Inc., Chicago, IL, USA).

Results

Table 1 shows the number of G6PD deficient patients, the number of all screened patients, and the percentage of normal enzyme activity in erythrocytes of all patients, both males and females. Seventy males (Gd−) and nine females (Gd−/Gd− and/or Gd−/Gd+) were diagnosed as G6PD deficient. The measured incidence was 1.6‰ in males, 0.2‰ in females and 0.9‰ in total newborns. The estimate incidence was 3.2‰ in females and 4.8‰ in total newborns. The incidence of missed females with G6PD deficiency was 93% of total females expected by Hardy–Weinberg law, and most of them are very likely heterozygote females.

The incidence of G6PD deficiency in Caucasian neonates in Veneto region, is lower than in Sardinia [16] and in other European regions [5, 17, 18]. However, the estimated incidence of G6PD deficiency is very likely similar to the incidence reported for Croatia (Table 2).

Discussion

According to the Hardy–Weinberg law, our results show that a high number of heterozygote G6PD (Gd−/Gd+) females are missed with qualitative screening method. It is not surprising that most of the heterozygote females belong to the intermediate deficient group and not to the severely deficient group. In such cases, the most accurate method to detect heterozygous G6PD females is mutation analysis (if known) in genomic DNA [19]. Unfortunately, this practice is not suitable for neonatal screening protocols.

One of the possible reasons for the different prevalence of the disease between populations is the negative and positive environmental selective pressure (such as the eradication of malaria and the decreased cultivation and consumption of vicia faba) [20]. Moreover in northeast Italy, immigration and intercultural marriages might suggest the lower incidence than in Sardinia and in Greek isles.

The gene for G6PD is on X-chromosome. Simple diagnostic tests have allowed precocious identifica-

Table 1. Incidence of G6PD deficiency in north-eastern Italy in newborns screened between years 2000 and 2001, measured and expected by Hardy–Weinberg equation

Neonatal screening in Veneto region	♂	♀	♂ + ♀
Positive/total screened (n°/n°)	70/43, 966	9/41, 471	79/85, 437
Enzyme activity in positives (U/g Hb)	2.52 ± 2.00	3.62 ± 2.62	2.65 ± 2.09
Measured incidence			
Frequency (q‰)	1.592	0.217	0.925
Expected incidence			
Homozygous (q^2‰)	–	0.003	–
Heterozygous ($2pq$ ‰)	–	3.179	–
Total (‰)	–	3.182	4.774
Δ (expected − measured) (‰)	–	2.965	3.849
Newborns loss (%)	–	93.2	80.6

Table 2. Incidence of G6PD deficiency in different areas of Europe

Areas	Subjects screened (n°)	Method of screening	Incidence (%)	M/F (ratio)	Ref.
Sardinia (Italy)	6187	Methaemoglobin reduction test	9.8	0.8	[16]
Greek	1,286,000	Fluorescent spot-test	3.14	2.5	[5]
Menorca (Spain)	1139 (males)	Methylene blue	0.97 (males)	–	[17]
Croatia	2726	Fluorescent spot-test	0.44	4.9	[18]
Veneto (Italy)	4706	Fluorescent spot-test	0.18	7.8	–

tions of all deficient males, with some difficulty in the diagnosis of transfused neonates. However, the predictive value of the screening tests is not well known, while the genotyping method is a useful tool for the confirmation of G6PD diagnosis. As previously reported [19], the recognition of heterozygote females was very difficult because their measured incidence was much lower than expected (4.2% of total). It is not unusual that heterozygote females with a severe deficient allele (Gd−) have a level of enzyme activity falling into the normal range [21]. An epigenetic phenomenon (lyonization) might explain these results. Therefore, females may show normal phenotype due to a skewed *X*-chromosome inactivation, so that survival and growth of G6PD normal cells might occur, and G6PD heterozygote females are missed from the screening procedure. We do not have information concerning the issue about the frequency of non-penetrating males, who carry the deficient allele but do not express the disease status.

In conclusion, a high proportion of G6PD heterozygote females are not detected by the screening program because they show a normal G6PD activity at birth. We can speculate that G6PD heterozygote females might show *X*-inactivation of deficient allele at birth, reduced lifespan of red blood cells, or both. The sensitivity of the fluorescent method is not sufficient to detect all heterozygote females. Since missed heterozygote females might develop the symptoms of G6PD deficiency later, these results suggest that current G6PD neonatal screening might not be helpful in preventing disease in females.

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