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Neonatal Screening for G6PD Deficiency in Mazandaran Province, Iran 2007-2010

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Abstract

Background: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common disease of the hexose monophosphate pathway existing in more than 400 million people worldwide. The aim of this study was to identify neonates with G6PD deficiency following national program for screening and education of affected newborns' parents started since June 2007 in Mazandaran, a northern Province of Iran.

Materials and Methods: Blood sampling was performed via a heel prick prepared for screening of congenital hypothyroidism, Phenylketonuria, and G6PD deficiency. Fluorescent spot test kit set by Kimia Pajohan Company (Iran) was used. A confirmatory test with a venous blood sample was done within 4th month of age according to the protocol. The second enzyme activity test was a quantitative photometric method using a commercially available kit (Randox, U.K.) with sensitivity of 154 IU and normal range of 6.97- 20.5 U/gHb. Data were analyzed using SPSS software version 16 and descriptive methods.

Results: During 36 months, 115622 newborns (51.4 % male) were studied. G6PD enzyme deficiency was found in 6.1% of the newborns (CI95%= 5.92-6.28%). As expected, male/female ratio of affected newborns was 6.19:1. Of confirmed affected infants in second enzyme activity test, 86.1% were male and 13.9% were female.

Conclusion: The reported rate of G6PD enzyme deficiency was less than expected (12-13%). We strongly recommend continuing screening and education of parents and also mass media education against consuming "Fava bean" and "Naphtalen".

Keywords: G6PD enzyme deficiency, Neonatal screening, Favism, Iran.

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency is an X-linked genetic disease and the most common enzyme deficiency in the world and Iran.[1] It is also the most prevalent enzyme deficiency in northern and southern provinces of Iran due to historical association with malaria outbreaks.[1,2] According to World Health Organization (WHO) classification, Iran is located in an area with 10-14.9% prevalence.[1] WHO recommends neonatal screening in areas with more than 3-5% prevalence in males.[2, 3] More than 400 different mutations related to G6PD deficiency have been recognized so far.[1-3] The most frequent mutations in Iran have been reported to be "Chatham", "Cosenza", and "nt 563(C_T)", so called "Mediterranean" mutations.[4-6]

The main clinical features include neonatal jaundice, and acute and sever hemolytic anemia in 2 to 6-year-old-children due to exposure to Fava bean (Favism).[7,8] The risk of Kernicterus is higher in these newborns. Moreover, Favism has its own complications and could lead to death. Other two clinical presentation forms, familial non-spherocytic chronic hemolytic anemia and drug induced hemolytic anemia, are rare and reportable in Iran.[7]

Neonatal screening and education for prevention of complications is constantly performed in some Mediterranean countries.[3,8] Screening methods include decolorization test, methemoglobin reduction test, and fluorescent spot test.[1] The third one is used in Iran. Screening is capable to reduce hospitalization rate caused by Favism.[9,10] This enzyme has a role in killing some bacteria especially

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Staphylococcus aureus, so deficient newborns have an increased risk of sepsis.[1] This aspect has not been covered in educational packages so far, while it is a recognized risk factor.[1,11] There are reports of delayed burn and traumatic wound healing in affected patients.[1] The program is managed by the Ministry of Health and Medical Education. Our goal was to identify neonates with G6PD deficiency through national program of screening and parents education started since June 2007 in Mazandaran, a northern province of Iran.

Material and Methods

This screening was performed during 36 months; from June 2007 to June 2010. As a screening program for congenital hypothyroidism and Phenylketonuoria, heel prick blood sampling was already in place, and the same blood was used for G6PD enzyme assay. The test was performed using Fluorescent Spot Test (Kimia Pajohan Company, Iran), and done in a local laboratory. The parents of affected patients were informed as soon as possible by a letter and an educational pamphlet. A confirmatory test using venous blood was also performed within 120 days of birth. The method was a quantitative photometric Enzyme Activity technique (kit Randox, U.K.) with sensitivity of 154 IU and normal range of 6.97- 20.5 U/gHb. Health workers made contact with the family by phone and encouraged them to do the confirmatory test. The first test was free of charge but the second one was paid by the parents and not covered by national insurances.[12] Descriptive statistics methods (frequencies) were applied using SPSS software version [16]. Confidence interval (CI) of 95% was calculated.

Results

During 36 months, a total of 115622 newborns (51.4 % male, 48.6% female) were studied. Coverage of the program was >100% because neonates of non-native women (travelers/guests) were also covered. Table 1 shows statistics for each year of the program. The rates of missing the confirmatory test were 39.3 % and 27.7 % in the first and second year, respectively (mean: 33.5%, CI 95%=32.1-33.9). Hence, the true positive rates of the first test were 60.6 % and 72.2 % (mean: 66.4%). As expected, male/female ratio of affected newborns was 6.1:1. Result of confirmatory test for the third year was not available at the time of writing this report. Of confirmed affected infants, 86.1% were male and 13.9% female. In other words, one in 25 boys and 1 in 69 girls are at risk for G6PD deficiency.

Discussion

Reported rate of G6PD deficient newborns in the first test, as well as, true positive rate were not acceptable and the reasons should be addressed. Mahdavi et al used florescent spot test for 1000 samples of cord blood with no confirmatory test. However, sensitivity of the test was assessed by double checking 100 samples using a quantitative method as a gold standard. The rate of the enzyme deficiency was reported to be 8.6% (CI95%=6.9–10.3) in all newborns, and 14.2 % (CI 95%=13.1-15.3) and 3% (CI 95%=2–4%) in boys and girls, respectively.7 Zhadedpasha et al used the same method in 1000 samples of cord blood, and reported that 12.5% of male newborns were enzyme deficient.8 This frequency was similar to the rate reported by Mahdavi et al.[7] Hashemi et al reported that 11.2% and 1.4% of male and female students were enzyme deficient in Amol.[13] In a published study by Mazandaran branch of Iranian Blood Transfusion Organization in 2009, 12.2% of 600 blood donors had G6PD enzyme deficiency. Their result is in accordance with the above mentioned studies.[7-8] Ahmadi et al reported that 13.6% of 1018 newborns admitted in a university hospital in Sari were G6PD deficient.[14] According to the previous reports,[7-8] and since their patients were admitted to the hospital because of high serum billirubin, the prevalence rate in the studied population was expected to be higher. Nabavizadeh et al evaluated samples from 261 donated blood bags and reported that about 14% of them were G6PD deficient.[15] As the majority of blood donors were male, this report is in accordance with Zahedpasha et al and Mahdavi et al.[7-8] In 2004, Abolghasemi et al assessed samples of donated blood of Tehran residents, and reported a 2.1% rate (3.6% and 0.6% in males and females, respectively) of the enzyme deficiency. This rate could be considered as a national prevalence rate because Tehran population (about 12 millions) is a mixture of all ethnic groups of the country. Since the enzyme deficiency seems to be rare in natives of dry central areas of Iran, the reported rate seems reasonable.[10] Behjati-Ardakani et al in a study on 454 newborns admitted because of high serum

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billirubin in Tehran reported that 7.6% had G6PD deficiency.16 Obviously, their sample was not representative of general population of Tehran, however, the reported rate for these high risk infants seems acceptable. Iranpour et al reported the result of newborn screening program in Isfahan. They quantitatively measured G6PD activity using a commercial kit of enzymatic colorimetric assay (GAMMA, Belgium) in which values less than 6.4 U/g Hb were considered G6PD deficient. Blood sampling was done by heel prick. They reported that 3.2% of all newborns (5.1% and 1% in males and females, respectively) were deficient. Mean (±SD) enzyme activity in deficient patients was 3.22±1.8 U/gHb (3.17±1.74 and 3.49±2.17 U/gHb in males and females, respectively (p=0.58)).17,18 Equal enzyme activity in both genders suggests that affected girls (explained by lionization phenomenon) are in the same danger as males. However, due to the x-linked inheritance, male/female ratio in affected subjects is always well above one.1 Although only 25% of Favism cases admitted in Sari hospitals were female, the severity of hemolysis was the same as males.[7]

Fluorescent spot test is a semiquantitative test. It is diagnostic in patients with less than 30% enzyme activity, and has rare false negative and few false positive (over-diagnosis) results. It is cheap and available, however, can not find all heterozygotes (females) and the sample should not be hemolysed. Quantitative test is expensive, not always available and even environmental temperature should be controlled at 30◦C.[1]

All mentioned references except Nabavizadeh et al used blood in its liquid form. But in national screening program, blood should be applied on filter paper and then resolved in a fixed amount of solvent. We have the experience of congenital hypothyroidism screening with the same method. At the beginning, false positive rate for hypothyroidism was inappropriately high (85%). The reason was a simple technical problem which was nearly resolved with more precise education and experience. Applying too much blood on the filter paper resulted in higher serum TSH levels (false positive), whereas, the same problem caused higher G6PD enzyme activity or false negative results. Report of Cohan et al from Shiraz supports this explanation. They reported that 237 (almost 80%) of 297 patients hospitalized with acute hemolysis and proved to be G6PD enzyme deficient, were falsely diagnosed as normal in neonatal screening. The main cause of hemolysis was ingestion of fava bean in 88.2%, underlying infection in 10.9%, and medicines in 0.8%. They measured enzyme level by a quantitative spectrophotometric method (Sigma, USA) on admission and 2 months later. They did not mention how they concluded that children were missed at screening; whether the laboratory results and/or health center records were controlled or they just asked mothers if they were informed.[9] Nevertheless, we do not agree with their suggestion to use quantitative tests because florescent spot test is cheaper and its low sensitivity is not proved. High false negative rate could not be explained merely based on sampling error. They mentioned that screening has decreased the hospital admission rate for Favism.9 Education and decreased birth rate may be considered as the most important reasons.

Table 1. Distribution of affected newborns according to the first and confirmatory tests for G6PD enzyme deficiency, Mazandaran, Iran, 2007-2010

Year of screening	No. of newborns	Positive in first test (%)	Positive in second test (%)	CI 95%	Percent of Male-Female (Ratio)
1	28884	1781 (6.16)	1080 (60.6)	3.5-3.9	86.6-13.4 (6.4:1)
2	38710	2385 (6.16)	1724 (72.2)	4.2-4.6	85.6-14.7 (5.8:1)
3	38028	2606(6.8)	NA	NA	NA

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Conclusion

We strongly recommend to continue neonatal screening and parents, as well as, general population education. However, research to find out the reason of under-diagnosis is needed. Either changing the sample to cord blood or collecting blood in liquid form by heel prick might solve the problem. Confirmatory test, if needed, should be postponed to the end of the first or second year of life, as venous blood sampling is less problematic and Favism is rare in infancy.

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