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Biochemical and cytochemical evaluation of heterozygote individuals with glucose-6-phosphate dehydrogenase deficiency

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Summary

The aim of this study was to diagnose heterozygous glucose-6-phosphate dehydrogenase (G6PD) deficient females by an inexpensive cytochemical G6PD staining method that is easy to perform, allowing diagnosis of G6PD deficiency without cumbersome genetic analysis. Three subject groups were included in the study. The first group consisted of 15 hemizygous deficient males. The second and the third group were composed of 15 heterozygous deficient females and 15 healthy individuals, respectively. Biochemical determination and cytochemical staining of G6PD activity were performed in samples of all subjects. Results obtained with the cytochemical staining method correlated significantly with the biochemical data ($p < 0.001$), but a only 51–68% of the erythrocytes were stained positively in females with normal biochemical G6PD activity despite their having a G6PD-deficient child. This observation clearly indicates that these individuals are heterozygously deficient. These findings show that the cytochemical staining method to detect G6PD activity in erythrocytes is reliable, sensitive and specific and is superior to the biochemical method. Therefore, this method can be used routinely to detect heterozygous G6PD deficiency.

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) (EC 1.1.1.49) is a housekeeping enzyme that catalyzes the first and rate-limiting step in the pentose phosphate pathway. Its key role in metabolism is to

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provide reducing power in the cytoplasm in the form of NADPH. This role is particularly important in red blood cells where NADPH is required for detoxification of hydrogen peroxide and other compounds, via reduced glutathione (Beutler, 1994; Lee et al., 1993). G6PD deficiency is the most common congenital enzyme deficiency in men, present in over 400 million people worldwide (Salvati, 1999). Human G6PD, a homodimer encoded by a gene which maps to the region Xq28 on the X chromosome, exhibits an extraordinary degree of genetic variability: more than 150 deficient variants have been reported and characterized (Beutler, 1990; Roos et al., 1999). Therefore, full manifestation of the defective gene is found in the male hemizygote and the female homozygote. In the female heterozygote, however, a mixed population (mosaic) of normal and enzyme deficient cells can be found. This is a result of random inactivation of one of the X-chromosomes (Lyon, 1961; Van Noorden et al., 1982; Gurbuz et al., 2004).

Hemizygously and homozygously deficient patients can be easily detected. However, heterozygously deficient females show G6PD activity levels in erythrocytes that are close to normal levels and are diagnosed as normal. Therefore, they cannot be detected by qualitative or quantitative biochemical methods. For the detection of these cases, a sensitive cytochemical staining method for G6PD activity in individual erythrocytes has been developed by Van Noorden et al. (1982), and is based on the reduction of a tetrazolium salt, tetranitro blue tetrazolium (TNBT), via an exogenous electron carrier, 1-methoxyphenazine methosulphate (1-methoxyPMS; Fig. 1), in a viscous medium containing polyvinyl alcohol (PVA; Van Noorden, 1984; Van Noorden and Vogels, 1985; Van Noorden et al., 1982). Such a cytochemical assay can be an excellent tool for discrimination between healthy persons and heterozygously, hemizygously and homozygously G6PD-deficient patients by the analysis of individual cells.

The aim of the present study was to diagnose heterozygous females with G6PD Mediterranean variant by this inexpensive cytochemical G6PD staining method that is relatively easy to perform. Our study differs from the previous studies, because the patient population in our study is different. G6PD is a very common disease in the Southern part of Turkey. In our country, we have Mediterranean variants of the G6PD deficiency (Akoglu et al., 1986; Aksu et al., 1990; Keskin et al., 2002). It is well know that G6PD variants have different characteristics. Therefore, we could not predict the results of this cytochemical method on Mediterranean variants. To the best of our knowledge, this is the first study performed in a Turkish population where different Mediterranean variants exist. Successful accomplishment of this specific aim allows diagnosis of G6PD deficiency without expensive and difficult genetic analysis.

Materials and methods

Subjects

- The hemizygous G6PD-deficiency group included 15 male patients who were previously diagnosed as deficient, and were between 0–20 years of age;
- The heterozygous G6PD-deficiency group included 15 females who are mothers of hemizygous patients. These subjects, within the age range of 25–45 years, were apparently healthy.

None of these subjects had ever experienced an acute hemolytic crisis;

- The control group was composed of 15 healthy subjects.

Written informed consent was obtained from all subjects.

Biochemical G6PD activity determination

Blood samples were obtained by venipuncture and collected in vacutainers containing heparin (15 IU/ml). Erythrocytes were separated by centrifugation at 800 g for 10 min and washed twice with isotonic saline. G6PD activity was determined by the ''modified Zinkham method'' in these packed cells (WHO, 1967). Enzyme activity was expressed as international units per gram hemoglobin (IU/g Hb).

Cytochemical staining method for G6PD activity

Blood samples were obtained by venipuncture and collected in heparinized tubes.

- In the first step, blood samples were incubated in 180 mM sodium nitrite for 8 min at room temperature for the conversion of all oxyhemoglobin into methemoglobin in order to prevent nonspecific formazan production.
- In the second step, packed cells were incubated with 20 mM $NADP^+$ for 10 min at room