Erythrocyte Glucose 6-Phosphate Dehydrogenase Deficiency Frequency in Gaziantep, Turkey

Erbağçı A.B., Yılmaz N.

Department of Biochemistry and Clinical Biochemistry, School of Medicine, University of Gaziantep, Gaziantep, Turkey

Objective: Glucose 6-phosphate dehydrogenase (G6PD) deficiency manifests genetic polymorphism and prevalence of its varying among geographic regions and ethnic groups. G6PD deficiency is important in Gaziantep, Turkey because high deficiency prevalence was observed in Adana and Antakya, the neighbouring Mediterranean cities.

Methods: In this study, sera from 306 subjects (166 female, 140 male) between 1-80 years old were for erythrocyte G6PD activity with International Committee for Standardization in Hematology (ICSH) method. After excluding the outliers, 95% interpercentile interval was accepted as reference limit.

Results: In Gaziantep reference group erythrocyte G6PD activity limits for subjects over one year old are 6.4 - 13.2 U/ g Hb, 30°C. Seven subjects with low enzyme activities indicate that G6PD deficiency frequency is approximately 2.3 ± 1% in Gaziantep.

Conclusion: G6PD deficiency frequency is approximately 2.3 ± 1% in Gaziantep which is higher than the mean prevalence in Turkey.

Key words: Glucose 6-phosphate dehydrogenase, reference limits, deficiency frequency

Glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate; NADP oxidoreductase; EC 1.1.1.49; G6PD) catalyzes the rate-limiting step of the pentose phosphate pathway, providing reducing power to the cell in the form of NADPH (1). NADPH is important for erythrocyte primary anti-oxidant defense system function, i.e. glutathion reductase, catalase and NADPH dependent methemoglobin reductase (2-4). Acute hemolytic anemia associated with oxidative stress is the most characteristic clinical manifestation of G6PD deficiency. G6PD is also a subject of public health, because to avoid hemolytic crisis is possible with a preventive approach (5).

World distribution of G6PD deficiency manifests a geographic pattern with the highest prevalence in West Africa (21%), some Asian countries such as Thailand (11%) and countries surrounding the Mediterranean Sea including Arab countries and southern Europe (6,7). G6PD deficiency prevalence is 18% in Saudi Arabia, 11% in United Arab Emirates, 11% in Jordan, 3.14% in Greece and 8.8% in Sardinia (6,8-11). Kurdish Jews has the highest known frequency of this trait (estimated gene frequency: 0.65) indicating the ethnic relation (12).

In Turkey, a Mediterranean country with a variety of ethnic groups, overall G6PD deficiency prevalence was determined as 0.5% in 1965. With respect to geographic distribution in Turkey, G6PD deficiency prevalence is between 0-2.3% in northern, eastern and western Anatolia (13). In southern Anatolia, especially the Mediterranean costs, G6PD deficiency frequency quite vary even among villages. G6PD deficiency prevalence is determined as 0.5-10.4% in Çukurova region, 6.5-17% in Antakya and Tarsus and 9.2% in Antalya (13-15). High G6PD deficiency prevalence of neighboring Çukurova region (endemic for malaria) and Antakya (where Hittite Turks constitute most of the population) calls attention to this trait in Gaziantep.

Material and Method

In this study, 306 individuals (166 female, 140 male) living in Gaziantep, who applied to local health units were included. Subjects were between 1-80 years old and did not have prediagnosed hematological disease or complaints related to hemolytic crisis at the moment. To establish the reference limits, central 95% interpercentile interval was determined after excluding highly deviating outliers.

Three ml of blood from antecubital vein was collected into heparinized tubes and stored at +4°C until the analysis that was performed within three days. Erythrocyte pellets were prepared after centrifugation at 5000’ g, +4°C for 10 minutes and discarding the plasma followed by two washing steps with isotonic NaCl solution in the same manner. The erythrocyte suspensions were prepared by suspending 300 mL erythrocyte pellet in 250 mL isotonic NaCl solution. Hemolyzates were prepared with addition of 9 volumes of lysing solution consisting of 2.7 mmol/L EDTA and 0.7mmol/L b-mercaptoethanol, pH 7.0 (100 mg of EDTA disodium salt and 5 mL b-mercaptoethanol in 100 mL of water) to one volume erythrocyte suspension and the complete lyzis was confirmed with microscopic examination.

Hemoglobin concentration of the erythrocyte suspensions was determined with an automatic complete blood counter (Medonic CA 160, France).

Erythrocyte G6PD activity was determined with kinetic spectrophotometric method according to the ICSH at 30°C, with slight modifications and water blank that gives exactly the same results either with the reagent or the sample blanks

Accepted for publication: 04 July 2002
(16,17). The reaction mixture contained 100 mL Tris HCl buffer 1mmol/L pH 8.0 (one mol/L tris and 65 mL HCl in 100mL, the pH being adjusted to 8.0 with HCl); 100 mL MgCl, 100mmol/L and 100mL NADP 2 mmol/L and 570 mL distilled water. After addition of 30 mL of haemolysate, reaction mixture was incubated at 30°C for 10 minutes. Reaction was started by adding 100 mL substrates (D-glucose 6-phosphate 6 mmol/L) and was monitored against water blank for 150 seconds after a lag phase of 90 seconds at 340 nm in temperature controlled cuvettes at 30°C.

Data are presented as mean ± SD. Comparison of variables was performed with the student’s t test. Test for normal distribution was evaluated with the Kolmogrov-Simirnov test. Correlations were tested with Pearson correlation analysis. Two tailed p values <0.05 were considered significant. SPSS 9.0 (SPSS Inc, Chicago, Illinois, USA) program was used for statistical analyses and illustrations.

Results

Erythrocyte G6PD activity distribution of the reference sample group defined as a ratio of hemoglobin concentration is presented in Figure 1. Erythrocyte G6PD activity (U/g Hb) is non-gaussian (p: 0.001) with high kurtosis values (Table I).

Table I. Statistical data of G6PD activity distribution in the reference sample group.

<table>
<thead>
<tr>
<th>G6PD Activity (U/g Hb)</th>
<th>n: 306</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>8.8</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>2.3</td>
</tr>
<tr>
<td>Median</td>
<td>8.6</td>
</tr>
<tr>
<td>Mode</td>
<td>8.3</td>
</tr>
<tr>
<td>Kurtosis</td>
<td>12.0</td>
</tr>
<tr>
<td>Skewness</td>
<td>1.6</td>
</tr>
<tr>
<td>Test for normal distribution (p)</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Although G6PD activity distributions exhibit a unimodal pattern, possibility of non-homogeneity was sought. G6PD activities of the sex groups were compared and a correlation analysis of age and G6PD activity was performed (18). G6PD activity was not statistically different in female (Mean: SD, 9.0± 2.1 U/g Hb) or male (8.6± 2.4 U/g Hb) subgroups (Table II). There was not a correlation between ages of subjects and G6PD activity (r: 0.002, p>0.05) (Figure 2).

As neither a difference between sex groups (p>0.05) nor a relation with age (p>0.05) was observed, unique reference interval was established for subjects over one year old.

After excluding highly deviating values, central 95% interval was accepted as reference limits as frequently used and recommended by the International Federation of Clinical Chemistry (IFCC) (18). With this non-parametric approach, G6PD activity reference limits are established as 6.4- 13.2 U/g Hb, at 30°C (Table II).

Table II. G6PD activity of female and male groups (Mean± SD) and unique G6PD reference limits for both genders in Gaziantep population.

<table>
<thead>
<tr>
<th></th>
<th>FEMALE</th>
<th>MALE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39.4± 16.8</td>
<td>39.2± 20.1</td>
</tr>
<tr>
<td>G6PD Activity (U/g Hb)</td>
<td>9.0± 2.1</td>
<td>8.6± 2.4*</td>
</tr>
<tr>
<td>Lower Limit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper Limit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G6PD Activity (U/g Hb)</td>
<td>6.4</td>
<td>13.2</td>
</tr>
<tr>
<td>n: 306</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p>0.05.

Figure 1. Erythrocyte G6PD distribution in the Gaziantep reference sample group.

Figure 2. G6PD activity and the ages of subjects.
Seven (4M, 3F) of the 306 subjects were with G6PD activities below the lower reference limit. Three of the G6PD deficient male subjects were with zero activity. One male and three female subjects were with intermediate activities. Although defining this ratio within 95% confidence interval requires screening approximately 1100 individuals, this ratio indicates that G6PD deficiency prevalence is approximately 2.3±1% in Gaziantep and could aid further studies.

Discussion

G6PD activity histogram in Gaziantep reference sample group demonstrates a high kurtosis value. This finding is not surprising because this is true for most of the analyses and in accordance with the constituent identity of the enzyme (3,19).

It is known that hematological parameters are in a dynamic process in the first year of postnatal life, related to the relatively young red cell population and alteration in active erythropoiesis centers (4,20,21). For this reason, newborns and infants were not included to this reference interval establishment study. The G6PD activity distributions were confirmed to be homogenous and a unique reference interval was established for sex subgroups and for subjects over one year old, which is an expected finding as well (6,22).

G6PD activity reference values according to ICSH are as follows: Mean: 8.34, SD: 1.59 U/g Hb, 30°C. Our method is quite similar to ICSH method, thus to compare the regional values (Mean: 8.8, SD: 2.3 U/g Hb, 30°C) should be a true approach. Our slightly higher values could be due to a pseudoincrease of G6PD activity proportioned to lower hemoglobin values possibly related of high incidence of iron deficiency anemia and talassemia syndromes in our country and region (23). In the point of reference limits this increase becomes more evident because of statistical differences. ICSH reference limits are (Mean±2SD): 5.2 -11.5 U/g Hb and our regional reference limits are (central 95% interpercentile interval) 6.4 - 13.2 U/g Hb. This non-parametric approach should be preferred in regions with a low G6PD deficiency prevalence, because it does not neglect the asymmetry of a distribution (18,19).

A clinical hemolysis is suggested to occur with normal erythrocyte population lower than 50%. This is equivalent of 4.4 U/g Hb, for variants with zero activity. It is also known that this ratio is usually higher than 50% for heterozygote subjects (24,25). With respect to drawbacks of subclinical hemolytic crisis and a slight chronic hemolytic anemia, our lower reference limit, approximately 75% of the mean should be convenient for the patients and the clinicians.

Seven (4M, 3F) of 306 subjects were with G6PD activities below the lower reference limit. Of the G6PD deficient group three male subjects were with zero activity and the rest were with intermediate values. World Health Organization reported that G6PD Mediterranean is the most common variant observed in Turkey. In addition to the three male subjects with zero activity, three female subjects (assuming them heterozygous for G6PD deficiency) whose G6PD activities are between 2.9-4.6 U/g Hb can be supposed to have G6PD Mediterranean variant. But the male subject whose enzyme activity is 3.7 U/g Hb indicates that other variants also exist in Turkey (27).

Seven individuals with low G6PD values of the reference sample group (n: 306) indicates that G6PD deficiency prevalence is approximately 2.3±1% in Gaziantep which is higher than the mean prevalence for Turkey (~0.5%). Defining this ratio within 95% confidence interval requires investigation of approximately 1100 individuals. However present data could aid further studies.

In conclusion, Gaziantep has distinct geographic, climatic, ethnic characteristics from neighbor Mediterranean region and this affects G6PD deficiency frequency, only slightly higher than the overall prevalence of Turkey.

References


Correspondence:
Dr. Ayşe Binnur Erbaççı
Department of Biochemistry and Clinical Chemistry,
Faculty of Medicine, University of Gaziantep,
TR-27310 Gaziantep, Turkey
Tel: +90 342/3606060
Fax: +90 342/3601617
E-mail: erbagci@gaziantep.edu.tr