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

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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, 30ºC. Seven subjects with low enzyme activities indicate that G6PD deficiency frequency is approximately 2.3 ± 1% in Gaziantep.**

Conclusion: **G6PD deficiency frequence 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, ie. 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).

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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 +4ºC until the analysis that was performed within three days. Erythrocyte pellets were prepared after centrifugation at 5000´g, +4ºC 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 suspensing 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 30ºC, with slight modifications and water blank that gives exactly the same results either with the reagent or the sample blanks

(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 $MgCl_2$ 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 subsrate (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.

	G6PD Activity (U/g Hb) n: 306
Mean	8.8
Standard Deviation	2.3
Median	8.6
Mode	8.3
Kurtosis	12.0
Skewness	1.6
Test for normal distribution (p)	0.000

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 (IFFC) (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.

	FEMALE n: 166	MALE n: 140
Age (years)	39.4± 16.8	39.2± 20.1
G6PD Activity (U/g Hb)	9.0± 2.1	8.6± 2.4*
	Lower Limit	Upper Limit
G6PD Activity (U/g Hb) n: 306	6.4	13.2

*p>0.05.

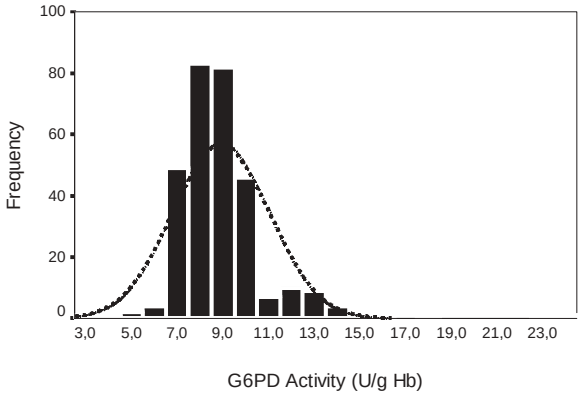

Figure 1. Erythrocyte G6PD distribution in the Gaziantep reference sample group.

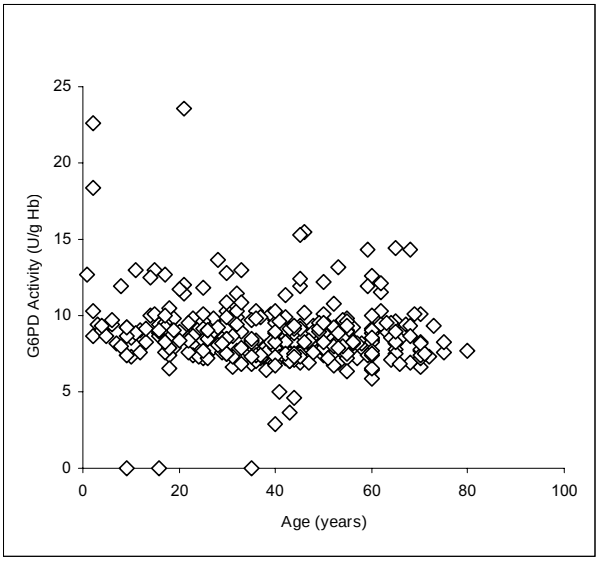

Figure 2. G6PD activity and the ages of subjects.

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 erythropoesis 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, 30^0C. Our method is quite similar to ICSH method, thus to compare the regional values (Mean: 8.8, SD: 2.3 U/g Hb, 30^0C) 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 prevalance 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

1. Naylor CE, Rowland P, Basak AK et al: Glucose 6-phosphate dehydrogenase mutations causing enzyme deficiency in a model of the tertiary structure of the human enzyme. Blood 87; 2974-2982, 1996.
2. Martinez Cayuela M: Oxygen free radicals and human disease. Biochimie 77: 147-161, 1995.
3. Geatani GF, Ferraris AM, Rolfo M, Mangerini R, Arena S, Kirkman HR: Predominant role of catalase in the disposal of hydrogen peroxide within human erythrocytes. Blood 87 (4): 1595-1599, 1996.
4. Nelson DA, Davey FR: Erythrocytic disorders. In: Henry JB, ed. Clinical Diagnosis and Management by Laboratory Methods. Philadelphia: WB Saunders Co, 1991, pp: 660-662.
5. Yüreir GT, Aksoy K, Arpac1 A, Ünlükurt 0, Tuli A: Studies on red cell glucose 6-phosphate dehydrogenase: evaluation of reference values. Ann Clin Biochem 31: 50-55, 1994.
6. Luzzatto L, Mehta A. Glucose 6-phosphate dehydrogenase deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D, Eds. The metabolic and molecular bases of inherited disease, USA: McGraw- Hill, 1995, pp: 3367-3398.
7. Tanphaichitr VS, Pung-amritt P, Yodthong S, Soonswang J, Mahasandana C, Suvatte V: Glucose 6-phosphate dehydrogenase deficiency in the newborn: its prevalence and relation to neonatal jaundice. Southeast Asian J Trop Med Public Health 1: 137-41, 1995.
8. Gelpi AP, King MC: New data on glucose 6-phosphate dehydrogenase deficiency in Saudi Arabia. G6PD variants, and the association between enzyme deficiency and hemoglobin S. Hum Hered 27: 285-291, 1977.
9. Bayoumi RA, Nur E, Kamal MS et al: Molecular characterization of erythrocyte glucose 6-phosphate dehydrogenase deficiency in Al-Ain District, United Arab Emirates. Hum Hered 46:136-41, 1996.

10. Talafih K, Hunaiti AA, Gharaibeh N, Gharaibeh M, Jaradat S: The prevalence of hemoglobin S and glucose 6-phosphate dehydrogenase deficiency in Jordanian newborn. J Obstet Gynaecol Res 22: 417-20, 1996.
11. Missiou T, Sagaraki S: Screening for glucose 6-phosphate dehydrogenase deficiency as a preventive measure: prevalence among 1,286,000 Greek newborn infants. J Pediatr 119: 293-299, 1991.
12. Oppenheim A, Jury CL, Rund D, Vulliamy TJ, Luzzatto L: G6PD Mediterranean accounts for the high prevalence of G6PD deficiency in Kurdish Jews. Hum Genet 91: 293-4, 1993.
13. Say B, Ozand P, Berkel 0, Çevik N: Erythrocyte glucose 6-phosphate dehydrogenase deficiency in Turkey. Acta Pediatrica Scandinavica 54: 319-324, 1965.
14. Akolu T, Özer FL, Çi S, Kumi M, Erdoan A, An1l H: Glucose 6-phosphate dehydrogenase deficiency in Çukurova Province, Turkey. Int J Epidemiol 10: 27-9, 1981.
15. Altay J, Yetgin S, Özsoylu S, Kutsal A: Hemoglobin S and other hemoglobinopathies in Eti- Turks. Hum Hered 28:56-61, 1978.
16. Luzzatto L: Investigation of hereditary haemolytic anaemias: membrane and enzyme abnormalities. In: Dacia JV, Lewis SM, eds. Practical Haematology Vol: 14, Singapore: Longman Singapore Pu. 1993, pp: 204-214.
17. Y1lmaz N, Erbac1 AB: Evaluation of erythrocyte G6PD activity unit; it is better defined as a function of RBC count. Gaziantep Tıp Dergisi 1,2: 58-63, 1999.
18. Solberg EH: Establishment and Use of Reference Values. In: Burtis CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry, Pennsylvania: WB Saunders Co, 1999, pp: 336-356.
19. Kahn SE, Jandreski MA. Laboratory Statistics. In: Kaplan LA, Pesce AJ, eds. Clinical Chemistry Theory, Analysis, Correlation. USA: Mosby Co, 1996, pp: 342-364.
20. Travis SF, Kumar SP, Paez PC, Papadoulos MD: Red cell metabolic alterations in postnatal life in term infants: glycolytic enzymes and glucose 6-phosphate dehydrogenase. Pediatr Res 14: 1349-52, 1980.
21. Vetrella M, Barthelman W: Enzyme activities in the erythrocytes of human fetuses. Z Kinderheilk 110:99-103, 1971.
22. Henry RJ, Cannon DC, Winkelman JV. Clinical Chemistry Principles and Technics. New York: Harper and Row Pu, 1974.
23. Aksoy M: Türkiye'de talaseminin tarihçesine k1sa bir bak1_. Çocuk Sal11 ve Hastal1klar1 Dergisi 34: 1-8, 1991.
24. Naumova AK, Plenge RM, Bird LM et al: Heritability of X chromosome-inactivation phenotype in a large family. Am J Hum Genet 58: 1111-9, 1996.
25. Panzion F, Zachello F, Sartori E, Addis S: The ratio between normal and sensitive erythrocytes in heterozygous glucose 6-phosphate dehydrogenase deficient women. Acta Haemat 43: 291-5, 1970.
26. Öner R, Acar C, Oner C, et al: Chronic hemolytic anemia associated with glucose 6-phosphate dehydrogenase (Guadalajara) 1 159 C® T (387 Arg ® Cys) deficiency associated with Gilbert syndrome in a Turkish patient. Pediatr Hematol Oncol 19: 39-44, 2002.

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