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The features of patients with favism in Turkey

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Abstract. Glucose-6-phosphate dehydrogenase deficiency (G6PD) in south of Turkey is a very important health problem; the incidence of G6PD was reported 5,4-20 % by the surveillance studies. Patients with favism are admitted to urgent hospital service after ingestion of vicia faba beans especially at the spring season. Aim of this study was to investigate patients with favism who had a history of consumed fava as clinical, haematological, biochemical and mutations. Fifty patients, aged 1-16 years (mean±SD: 5.94±4.54 years), 40 males (80%) and 10 females (20%) were included in this study. The complaints of them were pale (100%), icterus (84%), haemoglobinuria (72%), abdominal pain (60%) and fever (4%). In their history they had neonatal hyperbilurubinaemia (40%) repeated acute haemolysis (10%) and chronic non-spherocytic haemolytic anaemia (6%). G6PD activities were ranging from a complete deficiency in 10 (20%), moderate in 12 (24%), mild in 5 (10%) to normal levels in 23 patients (46%). Molecular studies of the patients were detected in 35 subjects (70%); only Mediterranean mutations were detected Z (563^T). Inonclusion, our patients with favism had the same complaints and clinical findings with other G6PD patients but their biochemical enzyme levels were heterogeneous and the incidence of Mediterranean mutation was high.

Key words: G6PD • favism • Mediterranean mutations • Turkey

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INTRODUCTION

Glucose-6-phosphate dehydrogenase deficiency (G6PD) was discovered in the 1950's[1], the clinical manifestations of G6PD began to be understood in 1960's, the degree of variability of this enzyme and the distinction of various biochemical variants was found in 1970's and the mutations were identified in 1980's[2]. The vast majority of individuals with G6PD deficiency are usually asymptomatic and throughout life the people affected are not aware of their genetic abnormality. These individuals may manifest some clinical conditions such as neonatal hyperbilurubinaemia, acute haemolytic anaemia (AHA) due to favism, drugs or infections and chronic non-spherocytic haemolytic anaemia in normal life period[3].

First surveillance studies on G6PD deficiency in southwest of Turkey were reported by Sipahioπlu, Aksoy and Aksu et al the incidence of G6PD deficiency found was 5,4-20%[4-6]. There have been no publications about patients with favism on clinical, haematological, biochemical findings and mutations in Turkey. Patients with favism were admitted to urgent hospital service due to different com-

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plaints after ingestion of vicia faba beans on spring season. Aim of this study was to investigate the patients with a history of consumed fava as clinical, haematological, biochemical and DNA mutations.

MATERIAL AND METHODS

A total 50 patients who had the history of ingestion of vicia faba beans were admitted to Antalya State Hospital. The mean age of patients were 5,94±4,54 years (range 1-16), 40 males and 10 females. The G6PD activity of the red cells was measured according to the modified Zincham method at Akdeniz University, Department of Biochemistry[7]. Molecular analysis of the patients was determined at Akdeniz University, Department of Molecular Biology. DNA was extracted from leucocytes, the polymerase chain reaction was performed to amplify the mutation at nucleotide 563^T of G6PD Mediterranean using by the primers AKA -CCC CGA AGA GGA ATT CAA GGG GGT-3') and AKB (5'-GAA GAG TAG CCC TCG ACT-3') in exon V-VI for G6PD Mediterranean563T. PCR was carried out with 300 ng sample DNA, 10 pmol each primer, 0,2 mM each dNTP(MBI Fermantes), 2 mM MgCl2, 1U Taq DNA polymerase (MBI Fermantes), in 50 ml total volume, at 94˚C/1 min denaturation, 56˚C/1 min annealing and 72˚C/1 min extension, in 30 cycle by Techne Progen thermal cycler. For 563^T mutation detection, 8 ml of the amplification product was digested with Mbo II (MBI fermentas) restriction endonuclease in 20 ml reaction volume at 37˚C and 54˚C at overnight, respectively. Digestion products were electrophoresed on 2,5% agarose gel ethidium bromide stained that was visualized under UV illumination[8-10].

RESULTS

The complaints of patinets were pale (100%), icterus (84%), haemoglobinuria (72%), abdominal pain (60%), and fever (4%). In their life history they had neonatal hyperbilurubinaemia (40%), repeated acute haemolysis (10%) and chronic non-spherocytic haemolytic anaemia (6%). On physical examination, there was anaemia in 50 of them (100%), icterus in 42 (84%) hepatomegaly in 2 (4%) and splenomegaly in 5 patients (%10). Haematologic findings were as following; Hb: 3,8-10.3 (mean±SD: 7,9±3,2) gr/dl, haematocrit:12,4-30,9% (mean±SD: 23,4±9,2) reticulocyte: 1-14% (mean±SD: 6,41±4,66); direct Coombs test was negative in all.

G6PD activity was found in a complete deficiency in 10 male patients (20%), moderate deficiency was found in 12 patients (24%), 10 males and 2 females, mild deficiency was in 5 male patients (10%); normal levels were found in 15 male and 8 female patients (Table 1).

Molecular study testing for Mediterranean mutation (563^T) was positive in 35 subjects (70%) (Table 2).

DISCUSSION

G6PD deficiency is genetically heterogenous. Over 400 different variants have been identified, and biochemical characterization indicates that it results from many allelic mutations in the G6PD gene[3]. Seven percent of the world population is affected from G6PD deficiency and it has occurred on an epidemic scale particularly in Mediterranean countries, Middle East, the Far East, North Africa[11]. In Turkey, the incidence of G6PD deficiency ranges from 0.9% to 20 % from region to region; different variants have been reported in the southern part of Turkey[4-6,12-17].

Genetic heterogeneity in G6PD deficient subjects explains the diverse clinical manifestations. All G6PD deficient subjects are not sensitive to fava beans[18]. When ^{51}Cr-labelled G6PD deficient cells from individuals with a clinical history of favism are infused in to normal subjects, challenge with primaquine always leads to haemolysis of deficient cells whereas challenge with fava beans leads to haemolysis only in some cases[19]. One or more factors in addition to G6PD deficiency are required for development of favism[20]. Interpersonel variability in a genetic component[21], low activity of erythrocyte acid phosphatase[22], decreased urinary D-glutaric acid and defec-

Table 1. The results of G6PD activity of the patients with favism (n:50)

G6PD	Male		Female		Total	
(IU/gr Hb)	n	%	n	%	n	%
0,0	10	20	-	-	10	20
0,1-3	10	20	2	4	12	24
3,1-5,0	5	10	-		5	10
5,1-7,4 (Normal levels)	15	30	8	16	23	46

Table 2. Mutation (563^T) analysis of the patients with favism(n:50)

Mutation (563^T)	Male		Female		Total	
	n	%	n	%	n	%
Pozitif	27	54	8	16	35	70
Normal	13	26	2	4	15	30

tive hepatic glucuronide formation[23], inversion of the peripheral blood helper T cell (CD4) to suppressor T cell (CD8) ratio[24], have been reported in favism patients. G6PD activities of our patients with favism were found heterogenous; this may be depended on the above factors.

Clinical favism presents characteristically with sudden onset of acute haemolytic anaemia within 24 to 48 h ingestion of the fava beans. The highest incidence occured in children aged 2 to 6 years, boys are affected two or three times more frequently than girls because of the greater number of hemizygous males than homozygous females. Favism occurs after ingestion of fresh, freed or frozen beans, but fresh beans are by far the commonest offender; therefore favism is commonest during the spring season[25]. Mean age of our patients were 5,94±4,54 years and 80 percent of them was male. All of them were admitted to hospital within 48 hour after ingestion of fresh beans during the spring season.

Mutations on G6PD gene spread over three different regions (Africa, Far East and Mediterranean) according to occurrence, ethnicity and geography. Mediterranean mutation occurs under two different haplotypes such as Med 1 and Med 2 (563^{T} ve $1311^{T)}$. It has been reported that the incidence of Mediterranean mutation was 66,6% in North of Italy, 41,5% in South of Italy, 95,2% in Middle East, 100 % in Espanol and Azhkenazi Jews and 76,4% in Pakistani[26]. G6PD-Adana, Samandaπ and Balcalύ variants which are clinically and biochemically different variants were determined in Hukurova region (southern Turkey). Mediterranean mutation Med 2 (563^{T} ve $1311^{T)}$ was showed only in G6PD-Adana variant[15]. In our study, 70% of patients were 563^{T} mutation.

As conclusion, our patients with favism had the same of complaints and clinical findings with other G6PD patients but their biochemical enzyme levels were heterogeneous; the incidence of Mediterranean mutation (563^{T}) was 70%.

REFERENCES

1. Meletis J, Konstantopoulos K. Favism-from the “avoid fava beans” of Pythagoras to the present. Haema 2004; 7: 17-21. Beutler E. Study of Glucose 6 phosphate dehydrogenase: history and molecular biology. Am J Hematol 1993; 42: 53-58.
2. Luzzatto L, Mehta A. Glucose 6 phosphate dehydrogenase deficiency. In Scriver CR, Beaudet AL, Sly WS, Valle D (eds): The metabolic basis of inherited diseases. Mc Graw Hill έnformation Series Company, 1989, pp. 2237-2365.
3. Sipahioglu H. G6PD, Venom and hemolytic anemia. Lancet 1967: 2: 15.
4. Aksoy M, Guncag D, Erdem S. Survey on haemoglobin variants, beta-thalassemia,glucose-6-phosphate dehydrogenase deficiency and haptoglobulin types in Turkish people living in Manavgat, Serik and Boztepe (Antalya). Hum Hered 1980; 30: 3-6.
5. Aksu TA, Esen F, Dolunay MS et al. Erythrocyte glucose-6-phosphate dehydrogenase(1.1.1.49) deficiency in Antalya provience, Turkey: An epidemiologic and biochemical study. Am J Epidemiol 1990; 131: 1094-1097.
6. WHO. Standardisation of procedures for study of glucose-6-phosphate dehydrogenase. Techn Rep Ser 1967; 366: 5-53.
7. Saiki RK. Amplification of genomic DNA. In Innis MA, Gelfand DH, Sninsky JJΦ, White TJ (eds): PCR protocols: A guide to method and amplifications, Academic Press Limited, London, 1990, pp. 13-20.
8. Beutler E, Kuhl W, Fox M, Tabsh K, Crandall BF. Prenatal diagnosis of glucose-6-phosphate dehydrogenase deficiency. Acta Haematol 1992; 87: 103-104.
9. Beutler E, Kuhl W. The NT 1311 polymorphism of G6PD:G6PD Mediterranean mutation may have originated independently in Europe and Asia. Am J Hum Genet 1990; 47: 1008-1012.
10. WHO Working Group, Update: Glucose-6-phosphate dehydrogenase deficiency. Bullettin of WHO 1989; 67: 601-611.
11. Ozlsoylu S, Sahinoglu M. Haemoglobinopathy survey in an eti Turk village. Hum Hered 1975; 25; 50-59.
12. Altay C, Yetgin S, Ozsoylu S, Kutsal A. Hemoglobin S and some other hemoglobinopathies in Eti Turks. Hum Hered 1978; 28: 56-61.
13. Yuregir GT, Aksoy K, Dikmen N, Unlukurt έ. Heterogeneity of glucose-6-phosphate dehydrogenase in Hukurova, Turkey. Biochim Clin 1989; 13: 933-935.
14. Aksoy K, Yuregir GT, Dikmen N, Unlukurt I. Three new G6PD variants, G6PD Adana, G6PD Samandað and G6PD Balcali in Cukurova, Turkey. Hum Genet 1987; 76: 199-201.
15. Kilinc Y, Kumi M. Haemolytic crises due to Glucose-6-phosphate dehydrogenase deficiency in the mid-southern region of Turkey. Acta Pediatr Scand 1990; 79: 1075-1079.
16. Akoglu T, Ozer FL, Akoglu E. The coincidence of glucose-6-phosphate dehydrogenase deficiency and hemoglobin S gene in Cukurova Province Turkey. Am J Epidemiol 1986; 123: 677-680.
17. Kattamis CA, Chaidas A, Chaidas S. G6PD deficiency and favism in the island of Rhodes. J Med Genet 1969; 6: 286.
18. Vullo C, Panizon F. The mechanism of hemolysis in favism.Transfusion experiments with ^{51}Cr tagged erythrocytes. Acta Haematol 1961; 26: 337.
19. Sartori E. On the pathogenesis of favism. J Med Genet 1971; 8: 462.
20. Mareni C, Repetto O, Foreteleoni G, Meloni T, Gaetani GF. Favism: Looking for an autosomal gene associated with Glucose-6-phosphate dehydrogenase deficiency. J Med Genet 1984; 21: 278.
21. Bottini E, Lucarelli P, Agostino R, Palmarino R, Businco L, Antognoni G. Favism: Association with erythrocyte acid

phosphatase phenotype. Science 1971; 171: 409-411.
22. Cassimos CHR, Malaka-Zafiriu K, Tsiures J. Urinary D-glucaric acid excretion in normal and G6PD deficient children with favism. J Pediatr 1974; 84: 871-872.
23. Schiliro G, Sciotto A, Russo A et al. Lymphocyte changes in favism:In vitro evidence of a modifying effect of bilirubin and hemoglobin on T-lymphocyte receptors. Acta Haematol 1983; 69: 230-235.
24. Meloni T, Forteleoni G, Dore A, Cutillo S. Favism and hemolytic anemia in glucose-6-phosphate dehydrogenase deficiency subjects in North Sardinia. Acta Haematol 1983; 70: 83-90.
25. Bagci H. In subjects with glucose-6-phosphate dehydrogenase deficiency, determination of mutations by genetics engineering and enzyme variants by biochemical methods. TUBITAK Project: SBAG-1467, Final Report, Ankara, 1998.