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Regional distribution of glucose-6-phosphate dehydrogenase deficiency in Turkey and evaluation of clinical findings: a multicenter study

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ABSTRACT

Objectives: The single most inherited enzyme deficiency is that of glucose-6-phosphate dehydrogenase (G6PD) with a presence in almost 400 million of the world'spopulation. The number of reported G6PD mutations is 186. Furthermore, geographical location is a determining factor for the prevalence of G6PD. Therefore, much of the existing epidemiological literature concerning this issue in Turkey has reported data specific to cities and regions. The purpose of this study was to examine G6PD deficiency in a sample of subjects. Outcome measures reported in this study include the clinical factors associated with the deficiency, as well as in geographical dispersion across regional locations in Turkey.

Methods: This is a retrospective, cross-sectional study. The sample comprised 308 subjects with a G6PD diagnosis. Data collection commenced in January 2011, and was completed by May 2020.

Results: In Turkey, the Mediterranean region has the greatest prevalence of G6PD enzyme deficiency. Subjects presenting with this deficiency were also diagnosed with haemolytic anaemia that was attributed to favism. Subsequently, drug and neonatal hyperbilirubinemia-induced haemolysis ensued. Over 90% of subjects diagnosed with a critical G6PD deficiency and recurrent haemolysis were allocated to the Class II variant.

Conclusions: The Mediterranean, along with Agean and Marmara regions are where the highest prevalence of G6PD enzyme deficiency are observed. Favism-induced haemolytic anaemia is the most often identified clinical precursor to diagnosis of G6PD deficiency in Turkey. The most common clinical feature after this condition is drug related haemolysis and the onset neonatal hyperbilirubinemia.

Keywords: Favism, glucose phosphate dehydrogenase deficiency, hemolytic anaemia

The first enzyme encountered in the pentose phosphate pathway is Glucose-6-phosphate dehydrogenase (G6PD). It is also the most rate-limiting enzyme in the pathway. For RNA, DNA and nucleotide synthesis to take place in this same pathway, ribose-5- phosphate must also be produced. Another product of this pathway is nicotinamide adenine dinucleotide phosphate (NADPH), which increases reduced glutathione (GSH) and thus prevents oxidative stress [1]. Due to the fact that there are no mitochondria within the erythrocytes, the pentose phosphate pathway is the sole route of production of NADPH.

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Inhibition of G6PD restricts NADPH production and thus, when exposed to drugs and infections, erythrocytes are subject to oxidative stress.

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an inherited disorder. Men are mostly subject to G6PD deficiency due to its X-linked transmission. To date the number of reported G6PD mutations is 230, and it is present in 0.5 billion of the world's population-making it the most prevalent inherited enzyme deficiency [2,3]. Geographical dispersion is an influential factor in G6PD deficiency incidence, while ethnicity also impacts on the prevalence of the deficiency too. Findings concerning the geographical dispersion of plasmodium falciparum and G6PD deficiency are comparable. In patients with G6PD deficiency, there is an argument to support the effectiveness of plasmodium falciparum resistance [4, 5]. In 1989, the World Health Organizations (WHO) reported that one or more G6PD deficiencies were present in at least 5% of the world's population [2]. The most affected populations were those of sub-Saharan Africa with a prevalence of 15%, and Southeast Asia at 26% [2]. This contrasted with Turkey with a percentage ranging from 0.5% to 2.9%, while similar findings were reported in other countries in proximity to the Mediterranean Sea, as well as the United States of America [2].

The majority of existing epidemiological data collected in Turkey concerning G6PD deficiency has focused on geographical distribution across regions, with the greatest frequency being reported in Cukurova (5.8-8.5%), while other regional frequencies include the Aegean at 2.3%, and the Black Sea at 5% [6, 7]. Additionally, in those screened for neonatal hyperbilirubinemia, G6PD deficiency was present in 1.12% [8]. Due to the high prevalence of G6PD in other countries in the Mediterranean region, nearby countries to Turkey, such as Italy and Greece have incorporated G6PD deficiency screening into their standard screening programme for newborn children.

There is a general consensus that G6PD deficiency is unlikely to negatively impact on a patient's quality of life, or their life expectancy [9]. However, recently, a study has reported that the risk of cardiovascular disease is observed more frequently in men with G6PD deficiency than in the control group (odds ratio, 1.39; confidence interval, 1.04-1.87) [10]. Diagnosis of the deficiency is often not made until the patient is in the neonatal or paediatric phase due to its asymptomatic nature. For example, G6PD deficiency is often undiagnosed until after patients are presenting with neonatal jaundice, favism, non-spherocytic chronic hemolytic anaemia or acute hemolytic episodes, the onset of which can be triggered by infections, or the ingestion of chemicals or medication [3].

Lack of enzymes is the marker used to specifically diagnose G6PD deficiency, which can be assessed by qualitative fluorescent spot test, methemoglobin reduction test or quantitative spectrophotometric measurements. Although it is not a preferred diagnostic option, it is also possible to use the family screening method in prenatal diagnosis. This method utilises the polymerase chain reaction to identify specific gene mutations [11].

The purpose of this study was to examine G6PD deficiency in a sample of G6PD deficient subjects. Outcome measures included in the analysis were clinical factors associated with G6PD deficiency, and geographical dispersion across the country of Turkey.

METHODS

All subjects included in this study had been applicants between the dates of January 2011 and March 2020. Data were retrieved restrospectively from hospital patient files stored on the internal information system. Demographic data analysed included patient age, home city and medical history. Clinical histories prior to diagnosis of G6PD deficiency were questioned in the anamnesis of the patients and noted. The clinical measures from each patient included in the analysis were; complete blood count, serum AST, LDH, total and direct bilirubin levels. Quantitative spectrophotometric methods were utilised to measure G6PD levels - a reading of over 4.6 IU/gHb was recommended to be a normal value for G6PD levels by the testing kit manufacturer.

There are five variants of G6PD enzyme deficiency as described by WHO – all of which are characterised by enzyme activity levels and measures of clinical factors [2]:

Class I: Severe deficiency - presents as less than 10% activity alongside chronic (nonspherocytic) hemolytic anaemia (Chicagovariant).

Class II: Severe deficiency - presents as more than

10% activity along side intermittent haemolysis, and subsequent to infection, or administration of drugs or chemicals (Mediterranean, Mahidol variant).

Class III: Mild deficiency – presents as between 10 and 60% activity, and alongside haemolysis in the presence of stressors.

Class IV: Non-deficient variant with no associated pathologies.

Class V: Elevated enzyme activity with no associated pathologies (Hektoen variant).

The study protocol was approved by the Institutional Review Board of Etlik Zübeyde Hanim Training and Research Hospital (IRB no. 2020/79).

Statistical Analysis

Quantitative data are reported in values of mean ± standard deviation, numerical units and percentage distribution n (%). Descriptive statistical methods were used at statistical evaluation of the patients.

RESULTS

Data from 316 subjects was selected for analysis. Of these, 308 male subjects, each aged above 18 years old were included in the final data set, as 8 of the original sample did not present clinically significant measures to enable diagnosis of G6PD deficiency. Regional distribution of the subjects is presented in Table 1. The distribution of the cities where the cases were living, was given on the map in Fig. 1.

Table 1. Regional distribution of subject diagnosed with G6PD deficiency

Region	Number of Subjects n (%)
Mediterranean	113 (36.7)
Aegean	85 (27.6)
Marmara	50 (16.2)
Central Anatolia	33 (10.7)
Southeastern Anatolia	18 (5.9)
Black Sea	8 (2.6)
East Anatolia	1 (0.3)
Total	**308 (100)**

Table 2 presents sample data for age, hemoglobin, hematocrit, serum AST, LDH, total, indirect bilirubin and G6PD levels.

In 67 (21.8%) of the subjects, diagnosis of G6PD had followed one or more episodes of acute haemolysis, of which 41 had been attributed to drugs, 23 to infection and 3 to chemicals. Diagnosis of G6PD deficiency following favism-related haemolysis accounted for 184 (59.7%) of the subjects' diagnoses, while 39 (12.7%) followed haemolysis after prolonged neonatal jaundice. Family history of G6PD deficiency was the preceding factor to diagnosis in 18 (5.8%) of the subjects – all of whom had not previously experienced haemolysis. Table 3 presents the percentage dis-

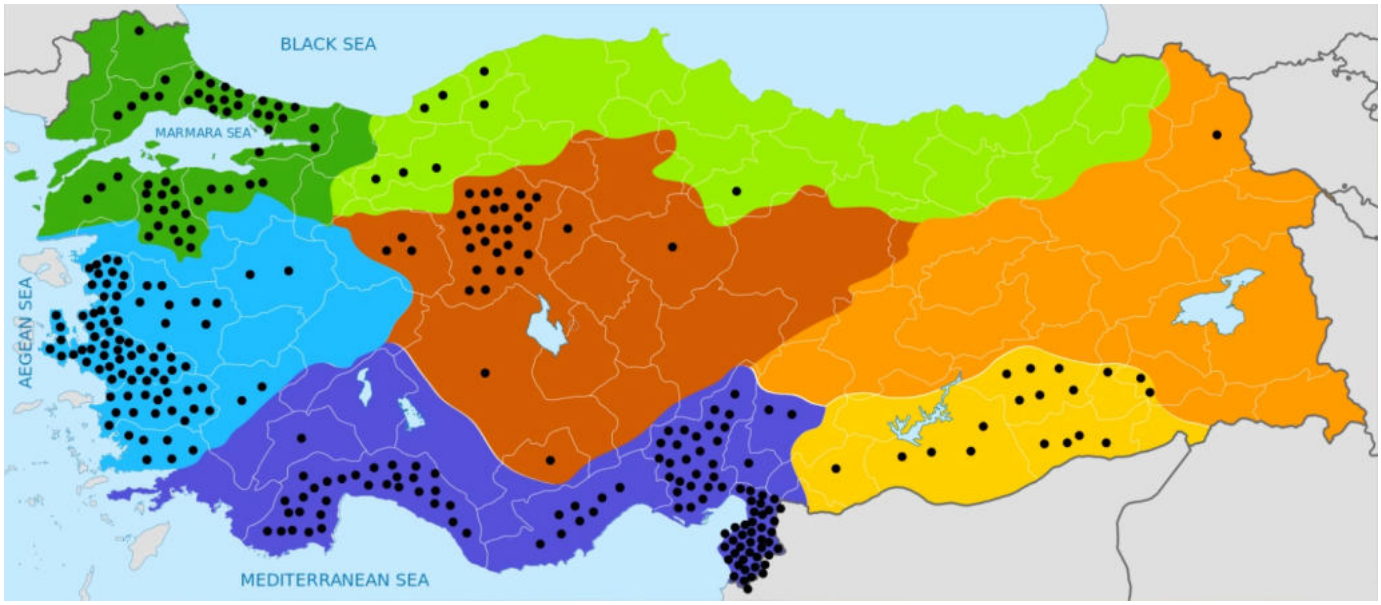

Fig. 1. Distribution of patients by geographical region/city (Every dots belongs to one patient).

Table 2. Age and laboratory data

Parameter	Unit	Mean ± SD
Average age	Year	25.23 ± 4.54
G6PD (4.60-13.50)	IU/gram hb	0.94 ± 1.00
Hemoglobin (11.6-17.0)	gr/dL	14.73 ± 1.44
Hemotocrit (34.9-50.1)	%	43.8 ± 3.76
AST (14-35)	U/L	24 ± 8.1
LDH (90-240)	U/L	251 ± 127
Total bilirubin (0.2-1.2)	mg/dL	1.44 ± 1.41
Indirect bilirubin (0.2-0.8)	mg/dL	0.27 ± 0.18

Table 3. Distribution of subject clinical history prior to diagnosis of G6PD deficiency

Clinical History	Number of cases n (%)
Acute hemolytic episodes	67 (21.8)
Drugs	41 (13.3)
Infection	23(7.5)
Chemical	3 (1.0)
Favism	184 (59.7)
Neonatal jaundice	39 (12.7)
Family history	18 (5.8)
Total	308 (100)

tribution of clinical precursors prior to diagnosis.

Analysis of the subjects' clinical presentations showed that 4 (1.3%) of G6PD deficiency in chronically haemolytic subject belonged to the Class I variant. In 271 subjects, G6PD deficiency was categorised as belonging to the Class II variant and 15 subjects belongs to Class III variant. There were 18 subjects whose G6PD deficiency could not be categorised due to an absence of haemolytic episodes, although each had a history of severe G6PD deficiency that was linked to a family history of G6PD deficiency.

DISCUSSION

There have been multiple studies conducted to examine the frequency of G6PD deficiency in numerous countries around the world, including Turkey. One or more gene G6PD deficiencies are present in almost 5% of the world's population, which means that around 500 million people are influenced by this deficiency [3, 12]. Geographical location and ethnicity of the population are both influential factors in the prevalence of G6PD deficiency. Jews represent the population with the greatest prevalence, with over 60% of this group having a G6PD deficiency, which is in contrast to the Pacific countries of Australia and New Zealand, where prevalence falls to as little as 0.1% [13, 14].

The findings of Aksu *et al*. [15] identified that in the city of Antalya around 7.4% of the male population was G6PD deficient. The data reported here by the authors of this study demonstrated that prevalence was greatest in the Mediterranean region, with the Agean and Marmara regions placed second and third in terms of prevalence. As we expected, the rate of G6PD was found to be higher in the regions adjacent to the Mediterranean, and was observed at a lower rate in the neighboring regions. Interestingly, the prevalence of G6PD deficiency in both these regions was above the Turkish average of 2.9%. However, prevalence has been reported to be higher still at 6.9% in Balikesir and Canakkale of the Marmara region in a healthy sample of 1421, while populations in the Black Sea region have demonstrated prevalence of G6DP deficiency at around 5% [7, 16]. The research evidence reported here has shown lower prevalence of G6DP deficiency than values reported elsewhere. It also appeared that the subjects of this study had attempted to conceal their diagnosed G6DP deficiencies during their health screening processes.

Those diagnosed with G6PD deficiency do not always present with precursors of acute hemolytic anaemia, neonatal jaundice or chronic non-spherocytic anaemia. Despite the relevance to the Turkish population this is not an issue that has been reported in any other published research findings. As predicted, the most frequent clinical feature reported prior to diagnosis of G6PD deficiency was favism at 59.7% of all cases. Favism is the onset of haemolytic anaemia in the 24 to 48 hour period following ingestion of fava beans – most commonly occurring in children aged between 2 and 5 years old, it is not often a fatal condition [17]. Favism in G6PD deficient people is determined by their genetic make up; not every individual who has a G6PD deficiency has favism, but every individual with favism will be G6PD deficient [18, 19].

The data reported here shows that favism is most common in those with a Class II variant of G6PD deficiency.

The frequency of neonatal jaundice in cases of pre-diagnosis of G6PD deficiency has been reported as between 1.12% and 3.8% [8, 20, 21] which is in contrast to the prevalence of 12.7% reported in the findings here. Thirteen percent of patients who present with neonatal jaundice are later diagnosed with G6PD deficiency, and therefore particular attention must be paid to those newborn patients presenting with hyperbilirubinemia, as this may lead to more successful rates of diagnosis for G6PD deficiency. It has been shown that a G6PD-deficient newborn who develops jaundice has higher bilirubin at admission, requires more exchange transfusion and prolonged phototherapy, and associated with a longer hospital stay [22]. In order to address the high rates of G6PD deficiency in Turkey it is important that G6PD deficiency tests are incorporated into the standardized health screening processes deployed at birth, as they are already in countries such as Italy and Greece. Most critically, this is to prevent life-threatening episodes of haemolysis – it is especially pertinent given the high prevalence of G6PD deficiency in the Mediterranean region. The data reported here identifies that the third most likely cause of heamolytic anaemia prior to diagnosis of G6PD deficiency is the administration of drugs (13.3%), of which antibiotics and anesthetic products were the most frequently recalled, although not officially documented. In those where haemolysis was caused by chemicals, the subjects reported that the chemical ingested was naphthalene. Eighteen did not experience haemolytic episodes, and therefore were not classified to this variant of G6PD deficiency, although they were screened and consequently diagnosed with the deficiency due to a family history of deficiency. Every subject in this sample presented levels of G6PD enzyme no greater than 10%, while all subjects reported favism to be a feature in their family history.

Based on the relevant levels of G6PD enzymes and their clinical features, there was only four (1.3%) Class I G6PD deficiency identified from the sample of 308 subjects – which were diagnosed due to his presentation of neonatal jaundice, and presented reports of haemolysis. There were 271 subjects who presented with a ClassII G6PD deficiency. Most existing studies of G6PD deficiency conducted in Turkey focused on distribution in regions and cities. The subjects in this study had been provided with different diagnoses in different regions of Turkey in order to explain their episodes of haemolysis. This was prior to successful diagnosis of G6PD deficiency through this research study, which was made based on their low enzyme levels and clinical symptoms. As such, it is possible that, the prevalence of ClassI G6PD deficiency identified here could represent general prevalence of ClassI G6PD deficiency across the regions and cities of Turkey.

There are limited reports on the prevalence of G6PD deficiency in women, due to the necessity of genetic diagnosis in studies and the rarer clinical manifestations of G6PD deficiency in women. However, data from a study of 355 female participants from previously malaria endemic areas in Northeast Thailand showed that the prevalence of G6PD Deficiency was much higher than expected (18% by fluorescent spot test, 29.6% by quantitation of G6PD activity) [23].

Limitations

A key limitation of these findings is that the data were analysed retrospectively. Therefore it was not possible to gain other measures at the point of data collection that may have proven to be valuable, such as genetic data. Because homozygous female genotypes are extremely rare, this study was limited to male patients only, and it is a limiting factor that it could not be included due to the lack of a diagnosed female patient.

CONCLUSION

In conclusion, these findings have demonstrated a higher prevalence of G6PD deficiency in the Mediterranean, Aegean, Marmara and Central Anatolia regions respectively. Furthermore, the most common clinical symptom to present prior to diagnosis of G6PD deficiency in the Turkish population is favism-induced haemolytic anaemia. Other common clinical symptoms prior to diagnosis are haemolytic anaemia attributable to drugs, neonatal hyperbilirubinemia and infections.

Authors' Contribution

Study Conception: CB; Study Design: MY; Super-

vision: SS; Funding: MKK, MA; Materials: MY; Data Collection and/or Processing: SS, EK; Statistical Analysis and/or Data Interpretation: MY, GÖ; Literature Review: MY, GÖ; Manuscript Preparation: SS and Critical Review: MKK, CB, MA.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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