The Study of Children with High Risk of HIV with in G6PD Enzyme Deficiency

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Annotation
Human immunodeficiency virus (HIV)-infected individuals receive oxidant prophylaxis, so hemolysis may occur in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Genetic screening was carried out for Masalli area of Azerbaijan Republic for 23 school students and found different degree of enzyme deficiency for G6PD enzyme (activity between 0-60%). Enzyme preparations were made according with WHO requirements of erythrocytes from identified students with enzymatic activity deficiency, and were identified biochemically specific for three classes out of existing five: 13 students – the 2nd class, 6 students – the 3rd class, and 4 students – the 4th class. Complying to the 2nd class requirements, 24 family members of the school student F.N. (index patient) from Bedalan town were screened for G6PD enzyme deficiency , and 6 of them showed acute enzyme activity deficit (lower than 10%). DNA molecular analysis, isolated from blood of the F.N. index patient, was classified as the 2nd class of G6PD enzyme deficiency, and showed the substitution of Guanine nucleotide with Adenine in position 1178. As a result of the mutation in protein in the position 393, substitution of amino acids Arginine with Histidine [G6PD,1178 (G-A) Arg393His] takes place.
Keywords: G6PD, HIV, biochemical polymorphism, enzyme, mutation, children, prevention

1. Introduction

Glucose-6-phosphate dehydrogenase (G6PD: EC 1.1.1.49) is defined with gene high polymorphism. Enzyme has been identified more than 400 biochemical variants and one fourth of them differ with endimicity. One part of the G6PD enzyme abnormal variants are endemic forthe certain ethnic group, and another part is specific to another ethnic group. One group of people with enzymatic deficiency get hemolytic crisis from some medicines and another group of people from getting beans (favism) with food (11,15,16).
Biochemical varients mainly are clinically asymptomatic. Big portion of biochemical variants result in hemolytic anemias under the influence of chemicals. And small portion of variants leads to severe chronical non-sphericytar anemia (2).
G6PD enzyme gene is located in X-sex chromosome and is transfered from heterozygous mother to a son. In women, if one of two X chromosomes is inactive, clinics in heterozygotes becomes different. In case non-affected enzyme gene being inactive, the affected gene mainly appears in erythrocytes, and the clinic manifestations are the same as in male hemizygote patients (1,12,14).
According to the data of World Health Organization (1997), around 100 million of world population manifest G6PD enzyme deficiency (2,8).
Human immunodeficiency virus (HIV) is an infection that attacks the body's immune system, specifically the white blood cells called CD4 cells. HIV destroys these CD4 cells, weakening a person's immunity against opportunistic infections, such as tuberculosis and fungal infections, severe bacterial infections and some cancers.
Many factors can induce hemolysis in G6PD-deficient individuals. Because HIV-infected patients frequently receive oxidative medications and often develop anemia, awareness of G6PD deficiency is particularly important(13).
WHO recommends that every person who may be at risk of HIV should access testing. People at increased risk of acquiring HIV should seek comprehensive and effective HIV prevention, testing and treatment services. HIV infection can be diagnosed using simple and affordable rapid diagnostic tests, as well as self-tests. It is important that HIV testing services follow the 5Cs: consent, confidentiality, counselling, correct results and connection with treatment and other services(17)
For Azerbaijan Republic population, study of G6PD enzyme deficiency started in the 70s of the last century. Studies were satisfied with only enzymatic

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activity studies (3,4).
The last century 70s' studies undertaken for Masalli area population revealed G6PD enzyme activity deficiency high frequencies (20-23%). Thus, Masalli area was not a random choice when starting our studies (14).
The goal of our studies was to research of G6PD gene molecular genetic and G6PD enzyme physic-chemical characteristics for the index patient from school students living in Masalli area chosen from people with abnormal G6PD activities.
Although the aim of our research is to study G6PD enzyme deficiency in the blood, it is also to draw attention to the creation of other blood diseases as a result of the weakening of the immune system. Examples of this include anemia, HIV virus activity, and leukemia.

2. Material and Methods

Material was collected during screening from school students from Arabkendi, Gullutepe, Tekle, Chakhirli, Bedalan towns of Masalli area and school students of 7-11 grades from Masalli downtown. As a result of screening of 276 school students we found 23 boys with inherited hemizygous type of G6PD enzyme.
Material collected for biochwemical studies was venous blood samples in tubes with EDTA anticoaggulant (5).
G6PD enzyme activity was identified with modified fluorescence method. To make the analysis accurate and to identify the inheritance type, we involved their parents anad family members into the study. Totally, there were 302 blood samples processed (6).
Purification of enzyme preparations and their classification were done according to the WHO standardized methods (2).
Molecular analysis of the G6PD enzyme gene was carried out in CENTOGENE laboratories, Rostock, Germany (7).

3. Results and Discussion

HIV-infected, G6PD-deficient individuals may also have inherently worse outcomes.For example, decreased glutathione levels in HIV-infected individuals suggest that they experience chronic oxidative stress. Primary HIV infection also caused acute hemolysis in a G6PD-deficient patient. Furthermore, oxidative stress is associated with immunological dysfunction, potentially exacerbating HIV infection(13).
As a result of 23 students screening on G6PD enzyme, different proportion of enzyme deficiency (activity 0-60%) was identified. According to the data of World Health Organization (WHO) in 1967, G6PD enzyme activity deficiency (based on the lack) was divided into 5 classes: the 1st class – chronic non-spherocytar anemia; the 2nd class – acute deficiency of the enzyme (lower than 10%); the 3rd class – enzyme medium deficiency (activity 10-60%); the 4th class – very mild deficiency of the enzyme (60%) and the 5th class – lower range of normal enzymatic activity.
Our studies have revealed enzyme activity which complies with WHO 2nd, 3rd and 4th classes: 13 persons in the 2nd class, 6 boys in the 3rd and 4 students in the 4th classes.
In Table 1, results of the genetic screening of G6PD enzyme carried in Masalli area are presented. Table presents amount of people screened, phenotypic frequency, gene frequency of the deficit enzyme, classes according to the enzyme deficit and names of towns in Masalli area.
276 male school students and 24 their family members were checked up in five towns. G6PD enzyme phenotipic frequencies and gene frequencies were presented. High results were supplied for Masalli town school (11.11% and 0.1111 in d.f.). The lower results were for Arabkendiand Terle town students (5.56% and 0.0555 in d.f.). For the totalarea enzymatic defociency frequency was 8,33%, and gene frequency was equal to 0.0833.
G6PD enzyme deficit in Masalli regional center were classified as 2, 3 and 4 classes, in Gullutepe and Bedalan towns presented only the 2nd class, inArabkendi and Chakhirli - the 3rd class, in Tekle both the 2nd and 3rd classes were found. In Bedalan town hemizygote inheritance type for the enzyme was identified in F.N. index patient's 24 family members, and 6 different male persons with hemizygote inheritance type were found. The family tree is presented in Figure 1.

Table 1. G6PD enzyme genetic screening results for Masalli area school students

Place (town)	Amount of patients	Affected patients	Phenotypic frequency (%)	Gene frequency (in decimal fraction)	Enzyme deficiency based classes
Regional center Masalli	72	8	11,11	0.1111	2 students – class 2 2 students- class 3 4 students - class 4
Gullutepe town	38	4	10.53	0.1053	3 students - class 2 1 student - class 2
Arabkendi town	42	3	5.56	0.0555	3 students - class 3
Tekle town	54	3	5.56	0.0555	2 students - class 2 1 student - class 3
Chakhirli town	30	2	7.14	0.0714	2 students - class 3
Bedalan town	40	3	7.50	0.0750	3 students - class 2
F.N.(index patient) family members	24	6	25.0	0.2500	6 persons – class 2

Complying to the 2nd class requirements, 24 family members of the school student F.N. (index patient) from Bedalan town were screened, and 6 of them showed acute enzyme activity deficit (lower than

10%).

Table 2 presents physic-chemical characteristics of enzyme preparations made from blood samples of F.N. index patient and his six family members living in Bedalan town of Masalli area who were found to have enzyme deficit.

Table 2. Mutation variant of G6PD enzyme found in Bedalan town

Variant name	G6PD activity (%)	EP-mobility	K_m G6F mkmol	2dG6F utilization	pH optimum	Thermostability	Clinic manifestation
Bedalan	6.0-8.0	85-90	21.3-24.5	78.6-80.0	8.0-9.0	Weak low	Mild anemia

So, Bedalan variant is classified as a variant with enzyme low activity (6.0-9.0% from norm), for G6P substrate has low range of Km binding (24.4 mkm), for 2dG6P substrate analogue has high utilization degree (up to 80% of G6P substrate), and manifests mild anemia.

In 1991 Chen T.Y. studied 20114 nucleotide sequences involving G6PD enzyme gene. Amino acid sequences were decode by means of sequencing of G6PD enzyme gene.

Fusco et al. (2012) observed for 3 times Alu repeat gene at non-involved 51-translation part. 12 Alu genes are located in the biggest intron 2. Capellini and Fiorelli (2008) identified that enzyme G6PD consists of 515 amino acid bases (8,11,15,16).

In blood of F.N.(index patient), mutation of G6PD enzyme gene was identified as a result of DNA molecular analysis in CENTOGENE laboratories, Germany. Guanine nucleotide substitution with Adenine nucleotide was identified in position 1178. Mutation resulted in substitution of Arginine amino acid with Histidine amino acid in position 393.

For the first time, Filosa et al. (1992) found that the substitution of Guanine with Adenine nucleotide happens in position 1178, and authors agreed to name this new enzyme mutation as G6PD Portici. The authors related this new G6PD enzyme mutation to the second group according to WHO classification (9,10).

Thus, as a result of genetic screening in Masalli area living school students, 23 male students with different G6PD enzyme deficit (in 0-60% range) were identified. Complying to WHO requirements, the identified enzyme deficiencies were shared into three classes astotheir biochemical characteristics: 13 students –to the 2nd class, 6 students – to the 3rd class, and 4 students – to the 4th class. Complying to the 2nd class requirements, 24 family members of the school student F.N. (index patient) from Bedalan town were screened, and 6 of them showed acute enzyme activity deficit (lower than 10%). DNA molecular analysis of G6PD gene obtained from the blood sample of F.N. index patient identified substitution of Guanine nucleotide with Adenine nucleotide in position 1178. As a result of the mutation in protein in the position 393, there was substitution of amino acids Arginine with Histidine [G6PD,1178 (G-A) Arg393His].

4. Conclusion

1. G6PD enzyme deficiency for Masalli area has shown the following values: 8.33% for phenotypic frequency and 0.0833 (d.f.) for gene frequency.
2. According to the WHO requirements, and according to the biochemical characteristics of the identified enzyme deficiency, findings were related to three classes: 13 students to the 2nd class, 6 students to the 3rd class, and 4 students to the 4th.
3. Molecular analysis of G6PD gene identified substitution of Guanine nucleotide with Adenine nucleotide in position 1178. As a result of the mutation in protein in the position 393, there was substitution of amino acids Arginine with Histidine [G6PD,1178 (G-A) Arg393His].
4. Children suffering from Q6PD enzyme deficiency should be constantly protected from the risk of blood diseases, especially the HIV virus, and should be under regular medical supervision.

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