THE STUDY IN CHILDREN WITH G6PD ENZYME DEFICIENCY HIV RISK

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Abstract: Individuals infected with the human immunodeficiency virus (HIV) may be given oxidant prophylaxis, which can cause hemolysis in people with glucose-6-phosphate dehydrogenase (G6PD) impairment. A genetic screening of 23 school pupils in the Azerbaijan Republic's Masalli region revealed different degrees of G6PD enzyme impairment (with enzyme activity ranging from 0-60%). Enzyme preparations were then generated from the erythrocytes of these identified students with enzymatic activity deficiency in accordance with WHO requirements, separating them biochemically into three of the existing five classes: 13 students were classified as the second class, 6 students as the third class, and 4 students as the fourth class.
A G6PD enzyme deficiency screening was extended to 24 family members of the school student F.N., the index patient from Bedalan town, in accordance with the 2nd class requirements. Six of them had acute enzyme activity deficiencies (less than 10%).
A guanine nucleotide substitution with adenine at position 1178 in DNA extracted from the blood of the index patient F.N. revealed the 2nd class of G6PD enzyme impairment.

Key words: G6PD, HIV, biochemical polymorphism, enzyme, mutation, chıldren, prevention

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Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency stands as the most prevalent enzymopathological ailment among humans. This condition is characterized as a widely distributed, hereditary anomaly linked to the X-chromosome (Reclos et al., 2000). It is estimated to afflict roughly 400 million individuals globally (Noori-Daloii et al., 2004). G6PD (EC 1.1.1.49) is renowned for its considerable genetic polymorphism, with the identification of over 400 distinct biochemical variants, approximately a quarter of which exhibit regional prevalence. This condition is most commonly observed across vast regions encompassing Africa, Asia, and countries bordering the Mediterranean Sea (Frank, 2005). Some of these G6PD enzyme variants are endemic to specific ethnic groups, while others are specific to distinct populations.

Individuals with enzymatic deficiencies can be categorized into two distinct groups: one group may undergo hemolytic crises triggered by specific medications, while the other group may experience such crises upon consuming beans, a condition referred to as favism (Fuji H., et al. 1984, Xu W., et al. 1999, Zuo L., et al. 1999).

The majority of these biochemical variants do not exhibit clinical symptoms. However, a noteworthy portion of these variants can lead to hemolytic anemias when exposed to particular chemicals, while a smaller subset can result in severe chronic non-spherocytic anemias. The gene encoding the G6PD enzyme is situated on the X sex chromosome and is inherited from a heterozygous mother to her son. In females, the

clinical presentation among heterozygotes can vary, depending on which of the two X chromosomes is rendered inactive. When the unaffected enzyme gene becomes inactive, the affected gene primarily becomes evident in erythrocytes, resulting in clinical manifestations akin to those observed in male hemizygous patients (Hirono A., et al. 1995, Aghayeva S.A., et al. 2019).According to the World Health Organization's data from 1997, approximately 100 million people worldwide exhibit G6PD enzyme deficiency (Filosa S., et al. 1992)

Human immunodeficiency virus (HIV) is an infection that specifically targets the immune system of the human body, primarily impacting a type of white blood cells called CD4 cells. HIV inflicts damage upon these CD4 cells, compromising an individual's ability to defend against opportunistic infections, which may include tuberculosis, fungal infections, severe bacterial infections, and certain forms of cancer (Xu J.Z., et al., 2015).

Several factors have the potential to induce hemolysis in individuals with G6PD deficiency. Since HIV-infected patients frequently receive medications with oxidative properties and are susceptible to developing anemia, it is of utmost importance to be cognizant of G6PD deficiency in such cases (Xu J.Z., et al., 2015).

The World Health Organization (WHO) advocates that individuals who might be at risk of HIV infection should have accessible testing opportunities. Those at a heightened risk of contracting HIV should actively seek comprehensive and effective HIV prevention, testing, and treatment services. The diagnosis of HIV infection can be accomplished through uncomplicated and cost-effective rapid diagnostic tests, including self-tests. It is imperative that HIV testing services adhere to the 5Cs framework, encompassing consent, confidentiality, counseling, provision of accurate results, and facilitating the connection of individuals with treatment and additional support services (World Health Organization).

The principal aim of our investigation was to delve into the molecular genetics of the G6PD gene and the physicochemical attributes of the G6PD enzyme. We conducted this study using a selected index patient chosen from among school students residing in the Masalli region, characterized by atypical G6PD activity (Aghayeva S.A., et al., 2018).

While the central objective of our research centers on the scrutiny of G6PD enzyme deficiency in the bloodstream, it also aspires to enhance understanding regarding the potential emergence of other hematological disorders stemming from a compromised immune system. These disorders encompass anemia, the activity of the HIV virus, and the occurrence of leukemia.

Materials and Methods

We conducted data collection through screenings of school students hailing from various towns in the Masalli region, including Arabkendi, Gullutepe, Tekle, Chakhirli, Bedalan, and students in grades 7-11 from downtown Masalli. A total of 276 school students underwent screening, from which we identified 23 male individuals with an inherited hemizygous G6PD enzyme variant.

For our biochemical investigations, we procured venous blood samples and placed them in tubes containing EDTA anticoagulant, following the methodology outlined by Beutler (1998). The assessment of G6PD enzyme activity was accomplished using a modified fluorescence method. To ensure precision in our findings and to ascertain the inheritance pattern, we incorporated the participation of their parents and extended family members, leading to a total of 302 processed blood samples (Beutler, 1994).

The purification and classification of enzyme preparations adhered to standardized techniques recommended by the World Health Organization (WHO, 1988).

Results and discussion

Individuals who are both HIV-infected and have G6PD deficiency often experience more severe outcomes. This is attributed to their typically reduced levels of glutathione, which indicates chronic oxidative stress, further complicating their HIV infection. Moreover, primary HIV infection can trigger acute hemolysis in G6PD-deficient patients,

underscoring the intricate interaction between these two conditions (Julia Z. Xu et al., 2015).

In our research, we conducted screenings on 23 students to detect G6PD enzyme deficiency and identified varying degrees of deficiency, with enzyme activity ranging from 0% to 60%. According to the World Health Organization's 1967 classification, G6PD enzyme activity deficiency is categorized into five classes: the 1st class represents chronic non-spherocytic anemia, the 2nd class indicates acute enzyme deficiency (activity below 10%), the 3rd class signifies moderate enzyme deficiency (activity between 10-60%), the 4th class represents very mild enzyme deficiency (activity at 60%), and the 5th class denotes the lower range of normal enzymatic activity. Our study revealed that our participants predominantly fell into the 2nd, 3rd, and 4th classes, with 13 individuals in the 2nd class, 6 in the 3rd class, and 4 in the 4th class.

Table 1 provides an overview of the genetic screening results pertaining to G6PD enzyme deficiency in the Masalli area. The table encompasses information regarding the number of individuals screened, phenotypic frequency, gene frequency of the deficient enzyme, classification based on the degree of enzyme deficiency, and the names of towns within the Masalli region. Our study involved the examination of a total of 276 male school students and 24 of their family members, representing five different towns. It is noteworthy that the school in Masalli town exhibited a notably high prevalence of G6PD deficiency, with a phenotypic frequency of 11.11% and a corresponding gene frequency of 0.1111. Conversely, Arabkendi and Tekle towns displayed lower prevalence rates, with phenotypic frequencies of 5.56% and gene frequencies of 0.0555, respectively. Across the entire study area, the overall frequency of enzymatic deficiency was calculated at 8.33%, with a gene frequency of 0.0833.

In the regional center of Masalli, G6PD enzyme deficiency was categorized into the 2nd, 3rd, and 4th classes. Notably, Gullutepe and Bedalan towns predominantly exhibited the 2nd class, while Arabkendi and Chakhirli towns showed a prevalence of the 3rd class. Tekle town displayed occurrences of both the 2nd and 3rd classes. It is noteworthy that, in Bedalan town, we identified the hemizygote inheritance type for the enzyme deficiency in the 24 family members of the index patient F.N., alongside an additional six individuals with the same inheritance pattern (see Table 1).

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

Place (town)	**Amount of patients**	**Affected patients**	**Phenotypic frequency (%)**	**Genefrequency (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
Gullutepetown	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

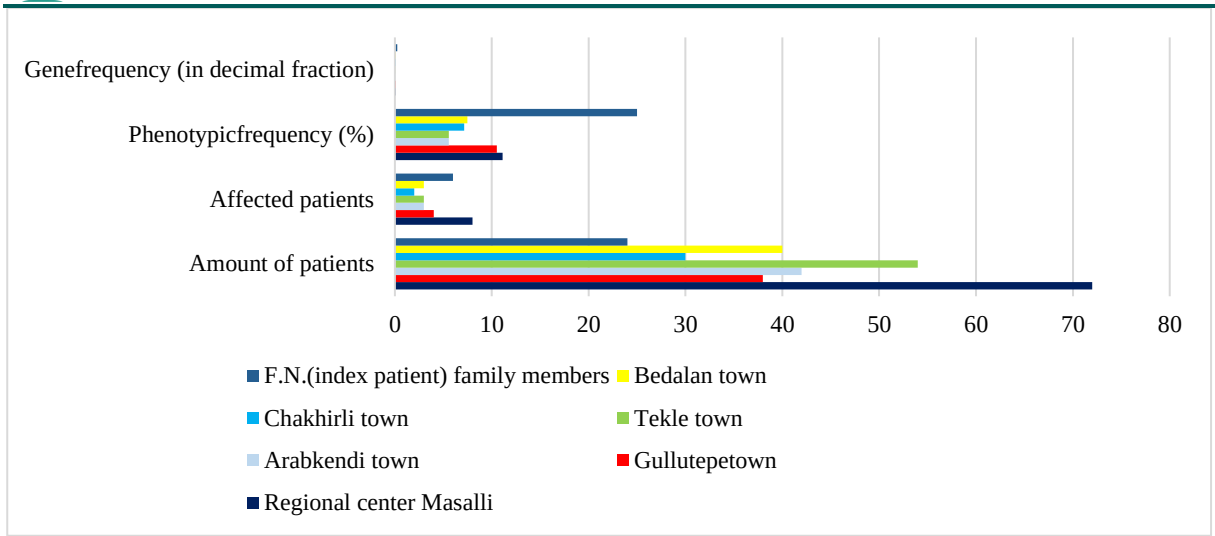

Fig 1.G6PD enzyme genetic screening results for Masalli area school students

Adhering to the standards established for the 2nd class classification, we conducted a comprehensive screening process for the 24 family members of the index patient, F.N., who is a student from Bedalan town. Among this group, six individuals displayed a notable deficiency in enzyme activity, with levels falling below the threshold of 10%.

Fig 1 is dedicated to presenting the physicochemical characteristics of enzyme preparations obtained from blood samples taken from the aforementioned index patient, F.N., and the six family members residing in Bedalan town within the Masalli region. It is important to note that all of these individuals were identified as having an enzyme deficiency.

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

Variant name	G6PD activity(%)	EP-mobility	K_mG6F 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

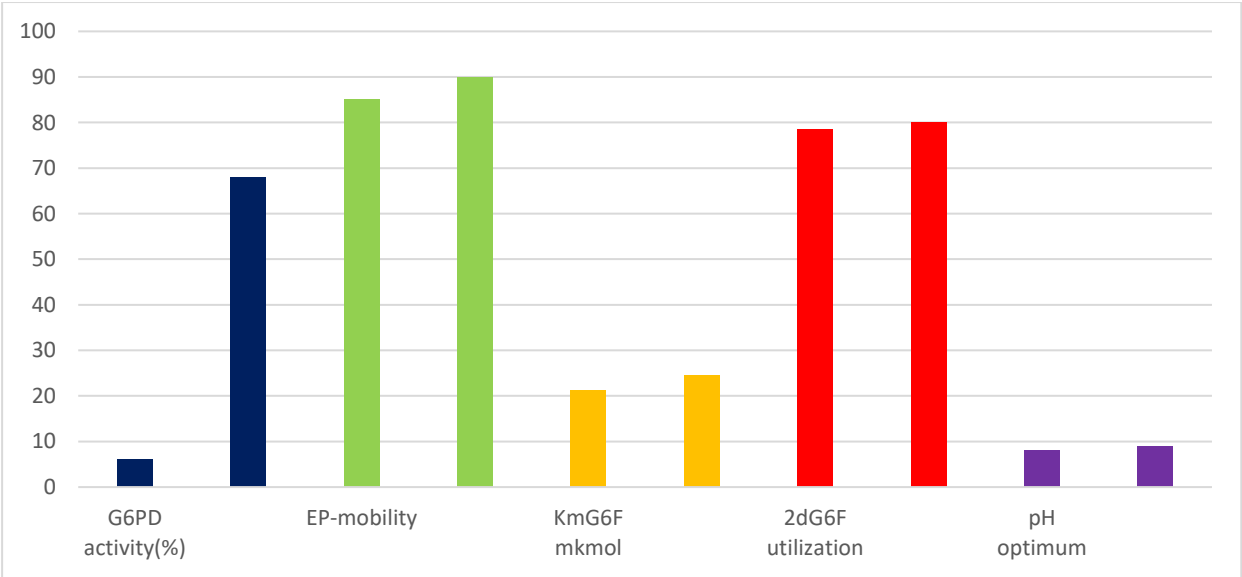

Fig 2. Mutation variant of G6PD enzyme found in Bedalan town

The Bedalan G6PD variant is classified as having low enzyme activity, typically ranging from 6.0% to 9.0% of the normal activity level. This particular variant is characterized by a low Km binding for the G6P substrate, measuring at 24.4 µM, and a notable degree of utilization for the 2dG6P substrate analogue, which can reach up to 80% of the utilization observed with the G6P substrate. Importantly, this variant is associated with mild anemia *(Fig 2).*

It's worth noting that there are over 400 distinct variants of G6PD that have been identified based on their biochemical attributes, enzyme kinetics, physicochemical properties, and other relevant parameters (Luzzatto B., 1985, Chen et al., 1991, Greene, 1993). The G6PD B+ variant is the most commonly encountered enzyme type and serves as the reference standard for normal enzyme activity and electrophoretic mobility. In the identification of other variants, G6PD B+ plays a pivotal role. The rate at which NADP+ is reduced by glucose-6-phosphate when G6PD B+ is the catalyst serves as the benchmark for activity measurement. Based on this benchmark, enzyme activities in relation to G6PD B+ variants are categorized as fast, normal, or slow with regard to electrophoretic mobility, and they are further classified into Classes I to V (Luzzatto, 1989, Beutler, 1990, Greene, 1993, Segel, 2000).

These categories encompass five distinctive groups: Class I is characterized by chronic non-spherocytic hemolytic anemia with a severe enzyme deficiency, illustrated by variants such as G6PD Minnesota, G6PD Tokyo, and G6PD Campinas. Class II variants exhibit a severe enzyme deficiency but do not manifest chronic non-spherocytic hemolytic anemia, as demonstrated by G6PD Mediterranean, G6PD Canton, G6PD Union, and G6PD Kaiping. Class III variants display moderate to mild enzyme deficiency, with activity levels ranging from 10% to 60% of G6PD B+, exemplified by G6PD A−. Class IV variants present minimal to no enzyme deficiency, with activity levels spanning from 60% to 100% of G6PD B+, typified by G6PD A+. Lastly, Class V variants show increased enzyme activity, with G6PD Hektoen serving as a notable example (Beutler, 1994, Segel, 2000).

Our investigation into the blood of the index patient, F.N., unveiled a mutation within the G6PD enzyme gene, as ascertained through DNA molecular analysis. This mutation entails the substitution of a guanine nucleotide with an adenine nucleotide at position 1178, resulting in the replacement of the arginine amino acid with the histidine amino acid at position 393.

Notably, Filosa et al. (1992) were the first to discover this substitution of a guanine with an adenine nucleotide at position 1178 and suggested the designation of this novel enzyme mutation as 'G6PD Portici.' According to the World Health Organization's classification, this new G6PD enzyme mutation is affiliated with the second group (Filosa S., et al. 1992, Filosa, S., et al. 1996, Du, C. S., et al., 1998).

The genetic screening of school students in the Masalli area led to the identification of 23 male students displaying varying G6PD enzyme deficiencies within the range of 0-60%. Following the guidelines established by the World Health Organization (WHO), we classified these identified enzyme deficiencies into three distinct classes based on their biochemical characteristics. Specifically, 13 students were categorized into the 2nd class, 6 into the 3rd class, and 4 into the 4th class.

In accordance with the criteria specified for the 2nd class, we conducted screening for the family members of the index patient, F.N., hailing from Bedalan town. Among these family members, six individuals exhibited acute enzyme activity deficits, measuring below the critical threshold of 10%.

Our investigation also involved a DNA molecular analysis of the G6PD gene extracted from the blood sample of F.N. This analysis unveiled a notable guanine-to-adenine substitution occurring at position 1178 within the G6PD gene. As a consequence of this mutation, the amino acid arginine was replaced by histidine [G6PD, 1178 (G-A) Arg393His].

Conclusion

Within the Masalli area, the presence of G6PD enzyme deficiency was observed at a phenotypic frequency of 8.33% and a gene frequency of 0.0833 (designated as d.f.).

In accordance with the stipulations set forth by the World Health Organization (WHO) and guided by the biochemical characteristics of the identified enzyme deficiencies, our findings were classified into three distinct classes. This categorization encompassed 13 students in the 2nd class, 6 students in the 3rd class, and 4 students in the 4th class.

Our molecular analysis of the G6PD gene unveiled a substitution event in which a guanine nucleotide was replaced by an adenine nucleotide at position 1178, leading to the alteration of amino acids from arginine to histidine [G6PD, 1178 (G-A) Arg393His].

It is imperative to ensure that children afflicted with G6PD enzyme deficiency receive consistent safeguards against the risk of blood disorders, with particular attention to conditions such as the HIV virus. Regular medical monitoring is highly recommended in these cases.

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