Frequency of G6PD (Glucose 6-Phosphate Deficiency) Anemia in the Patients Attending Peoples University Hospital Nawabshah.

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Abstract

Objective: To find out the frequency of glucose 6-phosphate deficiency anemia in the patients attending peoples university hospital Nawabshah.

Materials and Method: Type of study: Retrospective study. Sample size: 456. Duration of study: June 2017 to May 2019. Place of study: Nawabshah Sindh Pakistan. Sample collection and processing: Patient 5.0 mL blood samples were collected by venipuncture in an EDTA tube. To screen for G6PD deficiency using a fluorescence test, and using UDi G6P-DH kit from (United Diagnostics, Langerhagen, Germany) the anticoagulated whole blood was used. 5pl of anticoagulated whole blood was added directly into l00pl of working reagent, then mixed and incubated at room temperature for 10 minutes. After incubation, placed 10pl of resulting solution on the provided filter paper and waited to dry (15 minutes at 37°C) in a dark room, viewed the filter paper under a long wave UV light. Samples with normal or slightly deficient G6PD activity was shown fluorescence, while no fluorescence suggested complete lack or marked deficiency of G6PD.

Results: Total 456 patients were recruited from 1088. Out of them 203 were males and 253 were females. 35 females (13 83%) were positive for G6PD out of 253 while 218 (86.169c) were negative. 59 males (29.067o) were positive for G6PD out of 203 while 144 (70.93) were negative.

Conclusion: This research study found that G6PD deficiency is not so prevalent in the Nawabshah and its peripheries because only 20.61% showed positive results may be due to short duration of study as well as sample size.

Key words: Glucose 6-Phosphatase deficiency, Anemia, frequency.

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INTRODUCTION

In pentose phosphate pathway, the catalyzation of the first reaction (figure 1) is done by glucose-6-phosphate dehydrogenase (G6PD) enzyme that provides nicotinamide adenine dinucleotide phosphate (NADPH) (which is the reduced form of NADP) to all cells as a reducing power. Many oxidant factors can trigger an oxidative stress that counterbalanced by NADPH in the cells. Also, the NADPH allows glutathione reduced form to be preserved by the cells. The red blood cells defending against the oxidative stress is depending on G6PD due to lacking mitochondria, so the pentose phosphate pathway is their single source of NADPH."[2]

Mutations in G6PD gene cause glucose-6-phosphate dehydrogenase deficiency which is a hereditary metabolic, X-linked defect, leading to protein variants together with various levels of enzyme activity, which related to a broad range of biochemical and clinical

manifestations.G6PD gene length is 18 kb and it composed of 13 exons, it is located at X chromosome in the long arm (Xq28).[3] The locus of G6PD gene considered to be one of the utmost polymorphic loci between humans with nearly 400 allelic variants. There are 5 classes of variants which subdivided according to the functional severity of the deficiency; chronic non-sphero-cytic anemia due to severe deficiency is a characteristic of class 1 ranging to class 5 with more than 150% of natural activity. Hemolytic anemia and neonatal jaundice are the most prevalent manifestations that mostly triggered by exogenous factor. The noticeable similarity between the areas that Plasmodium falciparum malaria is endemic and G6PD deficiency is prevalent provides locative proof that G6PD deficiency gives resistance against malaria.[4]The greatest frequencies are identified in Mediterranean region, Asia, Africa, and in the Middle East; due to current migrations, nevertheless, the disorder is also found in North and in northern European countries and South America.[15] A large number of subjects having G6PD deficiency are mostly without symptoms. Acute anemia due to haemolysis and neonatal jaundice are the usual presentations in symptomatic subjects[6,7]. An infrequent fatal complication of neonatal jaundice is kernicterus which occurs in some particular populations [8].

Probably the most significant side to behold concerning the clinical manifestations of G6PD deficiency that it is remains asymptomatic throughout life. G6PD-deficient individual develops a disease only beneath certain conditions. In newborns, G6PD deficiency entails an elevated peril of neonatal jaundice (figure 3), involving severe neonatal jaundice.[9]

The reason for this has not been completely clarified, but it is considered as the most probable frequent cause of neonatal jaundice at countries where G6PD deficiency is widely spread, and it can lead to invalidating neurologic sequels if the proper treatment not taken. Therefore, sever complications can occur also in heterozygous G6PD-deficient baby girls, not only in hemizygous G6PD-deficient male babies.

On third day of this type of neonatal jaundice the bilirubin reaches the peak; this suggests that if the onset of neonatal jaundice is not taken earnestly as required, it may increases following discharging the baby. Ingesting fava beans (Viciafaba) (figure 4) can trigger acute hemolytic anemia in G6PD-deficient individual.[11]

This is due to presence of high concentrations of offending chemicals in fava bean which are nonvolatile glucosides (convicine and vicine), the aglycones of which produce free radicals.

The other beans are not containing these glucosides are harmless for G6PD-deficient individual. Favism can occur at any age, but it is widely prevalent and more frequently serious in children. The acute hemolytic anemia can be intense; a drop in hemoglobin from normal level to four g/dL can occur through 2 days or less, the intravascular hemolysis is indicated from associated macroscopic hemoglobinuria.

Many drugs have been considered to involve the risk of hemolysis in G6PD-deficient individual.[13]

As soon as the list of perilous drugs is recognized, any drug of listed should be eschewed in G6PD-deficient individual; drug-triggered acute hemolytic anemia is

significantly depending on the dose; thus, in exceptional cases when alternative is not available, a decreased dosage perhaps prescribed deliberately under proper supervision[14]

Laboratory Features

HematologyThe white blood cells increased during the attacks in G6PD deficient individual, while the platelets remain normal. The red blood cells during anemia are normocytic normochromic, and the reticulocyte count is elevated after crisis. (Figure5-B) Several abnormal red blood cells morphology may be seen in peripheral blood smear including Heinz bodies, polychromasia, degmacytes and occasional spherocytes. (Figure 5-A&C)

ChemistryThe lactic dehydrogenase and indirect bilirubin levels may be elevated, but haptoglobin level reduced through attacks.

Specific Tests Demonstration of low activity of the G6PD enzyme is required for diagnosis by screening test (e.g. fluorescent spot test) (figure 6) or by quantitative assay. (Figure1.6) Mutated specific gene which familiar to cause G6PD deficiency can recognize by molecular genetics examination, which is available exclusively at specialized laboratories[5].

Materials and Method

Type of study: Retrospective study.

Number of cases/sample size: 456.

Duration of study: June 2017 to May 2019.

Place of study: Nawabshah Sindh Pakistan

Sample collection: Patient five milliliter blood samples were obtained from venipuncture in an ethylene diaminetetra-acetic acid (EDTA) anticoagulated tube. The anticoagulated whole blood utilized to screen for G6PD deficiency by fluorescence spot test, using UD i G6P-DH kit from (United Diagnostics Industry, Langenhagen, Germany) as stated by the manufacturer's instructions. 5µl of anticoagulated whole blood was added directly into 100µl of working reagent, then mix and incubate at room temperature for 10 minutes. After incubation, place 10µl of resulting solution on the provided filter paper and wait to dry (15 minutes at 37°C). In a dark room, view the filter paper under a long wave UV light. Samples with normal or slightly deficient G6PD activity will show fluorescence, while no fluorescence suggests complete lack or marked deficiency of G6PD.

Results

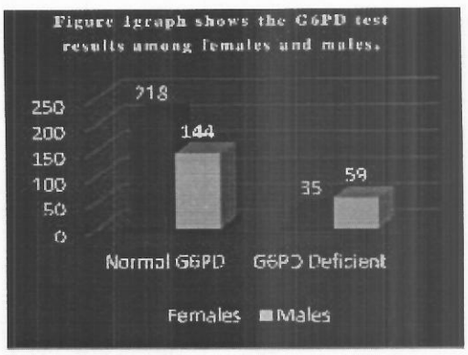
Total 456 patients were recruited from 1088 subjects from September 2017 to August 2019. Out of them 203 were males and 253 were females. 35 females (13.83%) were positive for G6PD out of 253 while 218 (86.16%) were negative. 59 males (29.06%) were positive for G6PD out of 203 while 144 (70.93) were negative.

Table 1

Gender	Normal G6PD	G6PD Deficient	Total
Females	218	35	253
Males	144	59	203

	G6PD Deficient	G6PD Normal	Total
Infants	21	69	90

Table 2shows the number of normal and deficient infants.



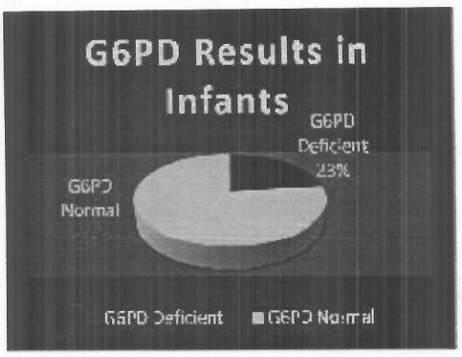


Figure 1 &2: shows the percentage of male and females, infants with G6Pd Deficiency among patients.

Discussion and Conclusion

G6PD deficiency is the commonest genetic disorder with red blood cell enzymopathy. This disease affects round about 4 million persons all around the globe [15]. Subjects with G6PD deficiency ailment are mostly reported from all areas of the globe but it is also observed that its prevalence is more in the areas where the plasmodium falciparum is most prevalent. Asia, Middle East, Mediterranean region and Africa are the regions where the occurrence of G6PD deficiency is highest [15].

This gene is expressed in all cells and is the maintenance gene. The major physiologic role of this is the provision of the NADPH, because the G6PD is the portion of pentose phosphate pathway. Deficiency of G6PD is one of the most common genetic enzyme anomalies in humans and is the universal health problem. It is caused by one of several

Probable mutations; the aging of red blood cells decreases the constancy and the level of the enzyme. Subjects with G6PD deficiency are usually asymptomatic. They are prone to develop severe jaundice in neonatal age. When these subjects use fava beans or exposed to certain medicines or had certain infections they develop severe hemolytic anemia[16].

In recent times, it was found that in subjects with acute myeloid leukemia (AML) experiencing intensive chemotherapy, and having G6PD deficiency are at risk for acquiring invasive fungal disease(IFD) especially candida sepsis. This indicates a crucial requirement to adopt appropriate strategies for management of subjects at increased risk of developing invasive fungal infections. So an algorithm is suggested for accurate documentation, prophylaxis, and management for subjects with invasive fungal infection and acute myeloid leukemia[17].

G6PD Glucose-6-phosphate dehydrogenase an enzymopathy of red blood cells affects about 08.00% of global populace mostly the affected regions are those where the malaria is endemic presently or in past.

Drug associated hemolysis and enhanced risks of fatal neonatal hyper bilirubinaemia are the major risks in the subjects with G6PD deficiency. The location of G6PD gene is on X chromosomes o the enzymatic deficiency due to mutations is observed in hemizygote males, homozygote females whereas intermediate activity is noted in a large number of heterozygous females ranging from 30% to 80% of normal, with a big range of

distribution between deficiency and routine.

The recent qualitative tests for G6PD are incompetent to diagnose the intermediate activities of G6PD; this can hamper the elimination of the Plasmodium vivax can be hampered by extensive use of 8-aminoquinolones [18].

Kingdom of Saudi Arabia which is scattered on area of 2149690 kilometers with a populace of 16.5 million, G6PD is reported from different areas of this country [19]. In current study the G6PD deficiency was noted in 94/456 (20.61%) of subjects, from these 13.83% (35/94) were female and 29.06% (59/94) were males. Current study is in arrangement with the earlier researches.

The sample size in current study is small so the findings of this study are restricted. Our outcome is required to be recognized by larger researches with further widespread molecular genetics categorization. The ecological distribution of G6PD deficiency equivalents with endemicity of malaria and about four hundred million subjects carry the abnormal gene universally [20]. Africa, Asia, Mediterranean countries and America are the countries where the prevalence of G6PD is higher. The global occurrence of this disease is thought to be due to shifting of population. In a meta-analysis the world wide occurrence of G6PD deficiency was analyzed as 04.5%, and prevalence in Pakistan was indicated as 01.8% [21].

Conversely, this meta-analysis unnoticed a number of locally printed non indexed documents in Pakistan that described occurrence of G6PD deficiency ranging from 02.00% to 04.00% in male subjects and the increased incidence up to 08.00% was reported in Pukhtoons [22,23,24,25].In two big national researches consisting of 1624 and 6454 babies admitted in hospitals for assessment of neonatal jaundice26.00% and 30.00% were reported to have the G6PD deficiency [26,27]. The major contributors of neonatal jaundice were documented as ABO or Rh incompatibility, Low birth weight and sepsis and in jaundiced babies G6PD deficiency was seen 08.00% [28].Malaria is endemic in Pakistan, with load of about 01.5 million cases yearly. In eradication of hypnozoites of plasmodium vivax and gametocytes of plasmodium falciparum the drug used is primaquine, conversely this drug causes hemolysis in subjects with deficiency of G6PD. The association between the malaria and the deficiency of G6PD is not significantly analyzed in Pakistan. The distribution of malaria and the deficiency of the G6PD should be outlined as to project efficient malaria abolition program [29].

Conclusion:

This research study found that G6PD deficiency is not so prevalent in the Nawabshah and its peripheries because only 20.61% showed positive results may be due to short duration of study as well as sample size.

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