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Categorization of Glucose 6 Phosphate Dehydrogenase (G6PD) Deficiency on the Basis of Enzyme Activity and its Clinico Haematological Correlation

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Author`s Contribution

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ABSTRACT

Objective: To categorize glucose-6-phosphate dehydrogenase (G6PD) deficiency based on enzyme activity and its clinical haematological correlation.
Methodology: This Cross-sectional study was conducted at the Department of Haematology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, from February 2022 to August 2022. Sampling was done using the nonprobability consecutive sampling technique. Test analysis included a complete blood picture, RBC morphology and reticulocyte count, G6PD quantitative test, and serum bilirubin. Thus, to categorize G6PDD based on its enzyme and clinic-haematological correlation, study included patients of both gender with an age ranging from 0-76 years. Descriptive statistics were expressed as mean ± standard deviation (SD) and categorical data were presented as frequency and percentage.
Results: Out of 120 study participants, 30 (25%) were females and 90 (75%) were males. The mean age of study participants was 10.83±12.75. G6PD PCR was detected among participants having G6PD deficiency level <1 U/g Hb and between 2-3 U/gm Hb. Hb levels below 8g/dL were found only in individuals with G6PD deficiency levels <1 U/gm Hb.
Conclusion: GDPD deficiency can be diagnosed by blood analysis comprising of complete blood count and RBC morphology aided by clinical correlation. The signs and symptoms increase in severity with a decline in GDPD enzyme function along with blood haemoglobin levels.
Keywords: Blood CP, Bite cells, G6PD, Heinz bodies, RBC morphology.

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Introduction

Glucose-6-phosphate dehydrogenase deficiency (G6PDD) is amongst the most common inborn error of metabolism.[1] As per American Society of Haematology, 500 million people (approx. 4.9%) worldwide live with G6PD enzyme deficiency.[16] Amongst the Middle East, Latin America, Africa, Asia, and the Mediterranean, the majority of G6PD enzyme deficiency lies in Asian countries due to high population density.[2,3] Besides, the global distribution of glucose-6-phosphate dehydrogenase deficiency is also related to the widespread occurrence of Plasmodium Falciparum malaria, as G6PD deficient patients may afford protection against malaria.[3,4]

The incomplete expression, absence, or reduction of the enzyme leads to the deficiency of G6PD, which can be inherited or acquired. The mutation in the G6PDX gene on the X-chromosome leads to the inherited X-linked recessive disorder, while ageing and conditions like metabolic syndrome can cause acquired deficiency.[5-7] Also, X-linked inheritance shows that the male population is affected mostly while the female population often remains carriers.[8] Initially, in 1950, the deficiency was found in some American soldiers who suffered haemolytic anaemia due to anti-malarial drugs. This means that symptoms develop and become worse after exposure to compounds such as oxidative stressors, e.g. fava beans,

oxidative medications (anti-malarial agents), and viral infections, e.g., COVID-19.[6,7]

The primary function of G6PD is to protect erythrocytes from oxidative stressors. G6PD status is essential as it modulates the level of reactive oxygen species by mainly producing nicotinamide adenine dinucleotide phosphate (NADPH) in the first step of the pentose phosphate pathway (PPP). NADPH plays an essential role in detoxifying reactive oxygen species (ROS).[2,9,10] Thus, the regular activity of G6PD deals with protecting RBCs from oxygen-derived oxidative stress,[10] whereas G6PD deficiency brings decreased NADPH production, which in turn makes the cells susceptible to oxidative damage and consequently red cell hemolysis. Mainly, extravascular haemolysis occurs, but intravascular haemolysis also happens to some extent and causes clinical symptoms of jaundice, anaemia, and black color urine.[1]

G6PD deficiency has been categorized into five classes based on clinical manifestations and enzyme activity. Class I represents the severe deficiency in correspondence with chronic non-spherocytic haemolytic anaemia (normal G6PD function <1). Class II shows residual activity associated with acute haemolytic anaemia (1-10% normal G6PD function). Class III demonstrates a mild form of mutation severity with 10-60%, Class IV shows normal activity with 60-150%, and Class V shows more than normal activity with the normal G6PD function >150%.[4]

As per a recent literature review, G6PDD categories are based on enzyme activity and clinical manifestations. The present study objective was to categorize G6PDD based on enzyme activity along with clinic-haematologic correlation and molecular studies were performed to assess the prevalence of the most common variant in our population, i.e. Mediterranean variant of G6PD and correlate it with its enzyme activity levels.

Methodology

This was a cross-sectional study, conducted at the Department of Hematology at the Armed Forces Institute of Pathology (AFIP), Combined Military Hospital, Rawalpindi. The duration of the study was six months from Feb 2022 to July 2022. Institutional Review Board (IRB) vide reference number (HEM21-22/READ/22/1444) provided us the ethical clearance. The literature review demonstrated a 2 to 8% prevalence of G6PD deficiency in Northern Pakistan. After a thorough literature search, a sample size of 114 was calculated using a WHO calculator, keeping a 5% margin of error, 95% confidence level, and prevalence of G6PD deficiency of 08%.[10] Sampling was done using the nonprobability consecutive sampling technique. Thus, to categorize G6PDD based on its enzyme and clinic-haematological correlation, our study included patients of both gender with an age ranging from 0-76 years. The patients were not only notified about the study but also an informed consent was taken from each. Written consent was taken from the guardian for the patient below the age of 15. A maximum number of available participants (120) during the study period were recruited.

Inclusion Criteria: Patients of all ages who presented for G6PD screening were included.

Exclusion Criteria: Patients with increased reticulocyte count, suffering from severe anemia (Hb 4-5 g/dl), and other haemolytic anaemias were not included.

A complete physical examination along with detailed history including drug and transfusion history was done. Test analysis included a complete blood picture (SYSMEX XN 3000), RBC morphology and reticulocyte count, G6PD quantitative test by the biosensor, and serum bilirubin (total and indirect). The index of the test was the basis of the G6PD Biosensor analyzer on the electrochemical principle of G6PD enzyme activity which measures electricity generated by the reduction of NADPH in accompanying ferricyanide. Out of 2ml EDTA blood sample taken from each patient, a 10µl sample was added to the extraction buffer cuvette which was then mixed and settled for 10 minutes. Then, the 10 µl sample was withdrawn from the pipette and applied to the specimen application hole of a test device. The results were obtained after 2 minutes. $\leq$4 U/g Hb cut-off value was kept as diagnostic of G6PD deficiency as the value is equal to <30% of adjusted median G6PD enzyme activity. The gold standard used for categorizing the G6PD deficiency was Trinity Biotech Quantitative Assay. At a wavelength of 340nm, the measurement of the change in absorbance determined the enzyme activity followed by a five-minute incubation at room temperature.

Data were entered in Microsoft excel and later analyzed using Statistical Package for Social Sciences (SPSS) 21.0. Descriptive statistics were expressed as mean ± standard deviation (SD) and categorical data were presented as frequency and percentage. A Chi-Square test was applied to determine the significant difference between different levels of G6PD Deficiency with clinico haematological parameters including G6PD PCR results, RBC

morphology, and haemoglobin levels. A p-value ≤0.05 was considered significant.

Results

Out of 120 study participants, 30 (25%) were females and 90 (75%) were males. The mean age of study participants was 10.83±12.75. The mean age of females was 7.54±9.51 years while the mean age of males was 11.92±13.52 years. The highest frequency of cases was noted in the age group 0-1 years as shown in Table I.

Table I: Gender distribution in different age groups.

Age Range	Males	Females	Total
0-1	42 (46.6%)	17 (56.7%)	59 (49.2%)
1-12	8 (8.9%)	3 (10%)	11 (9.2%)
13.25	19 (21.1%)	9 (30%)	28 (23.3%)
26-40	18 (20.0%)	1 (3.3%)	19 (15.8%)
41-60	3 (3.4%)	0 (0.0%)	3 (2.5%)
Total	90 (100%)	30 (100%)	120 (100%)

A chi-square analysis was performed to determine the significant difference between different levels of G6PD Deficiency with clinico haematological parameters including G6PD PCR results, RBC morphology, and haemoglobin levels. G6PD PCR was detected among participants having G6PD Deficiency level <1 and between 2-3. RBC morphology comprising bite cells, nucleated RBCs, spherocytes, Heinz bodies, polychromasia, and blister cells was present in the first three G6PD Deficiency levels groups. Hb levels below 8g/dL were found only in individuals with G6PD Deficiency levels <1 whereas the patients between the G6PD Deficiency levels of 2-3 had Hb levels between 8-10g/dL. Whereas individuals with G6PD Deficiency levels greater than 3 years had Hb levels of 10-16g/dL. (Table II)

Presenting complaint of pallor was most common, however severe pallor associated with symptoms of fever, pneumonia, malaria, and back urine were more prevalent with patients with less than 1 G6PD Deficiency level. Complaints of severe pallor along with anti-malarial treatment and malaria with black urine were most commonly associated with patients of the 2-3 G6PD Deficiency levels group. Details are shown in table III.

Discussion

Glucose-6-phosphate is a cytosolic enzyme that is found in the cell cytoplasm. It is known as a housekeeping enzyme because it prevents cell damage from reactive oxygen species. This enzyme helps in the protection of red blood cells in the body and their premature destruction. It helps red blood cells work and protect them from harmful substances. There are a series of chemical reactions

Table II: Clinical Parameters associated with G6PD Levels using Chi-Square Test.

Clinical Parameters		G6PD Levels					p-value
		<1	2-3	4-10	11-15	>15	
G6PD PCR	Detected	14 (11.7%)	10 (8.3%)	0 (0.0%)	0 (0.00%)	0 (0.0%)	<0.001
	Not Detected	10 (8.3%)	11 (9.2%)	42 (35.0%)	20 (16.7%)	13 (10.8%)	
RBC Morphology	Normal	0 (0.0%)	0 (0.0%)	24 (20.0%)	20 (16.7%)	13 (10.8%)	<0.001
	Bite cells, nucleated RBCs, Spherocytes, Heinz bodies, Polychromasia, Blister Cells	24 (20.0%)	19 0 (15.8%)	4 0 (0.0%)	0 (0.0%)	0 (0.0%)	
	Occasional Bite Cell, Polychromoria, Blister Cells	0 (0.0%)	2 (1.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	Occasional Bite Cell, Spherocytes	0 (0.0%)	0 (0.0%)	14 (11.7%)	0 (0.0%)	0 (0.0%)	
Hemoglobin	<8 g/dL	24 (20.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	<0.001
	8-10 g/dL	0 (0.0%)	17 (14.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	10-16 g/dL	0 (0.0%)	4 (3.3%)	42 (35.0%)	20 (16.7%)	13 (10.8%)	

*p-value was calculated by using the Chi-Square test.

Table III: Present Complaints associated with G6PD Levels using Chi-Square Test.

Present Complains	G6PD Levels					p-value
	<1	2-3	4.10	11-15	>15	
Severe Pallor, Fever, Pneumonia, Malaria, Black Urine	24 (20.0%)	0 (0.0%)	2 (1.7%)	0 (0.0%)	0 (0.0%)	<0.001
Severe Pallor, Anti-Malarial Treatment, Malaria, Black Urine	0 (0.0%)	21 (17.5%)	1 (0.8%)	0 (0.0%)	0 (0.0%)	
Suspicion of G6PD	0 (0.0%)	0 (0.0%)	39 (32.5%)	20 (16.7%)	13 (10.8%)	

occurring in our body that convert glucose (the sugar found in carbohydrates) to another sugar, ribose-5-phosphate, Glucose-6-dehydrogenase is responsible for the first step in this pathway of reactions.

G6PD is essential for RBCs to maintain their function. In the absence of this enzyme, exposure to any stress such as any drug, chemical, bacterial, or viral infection leads to hemolysis. A study conducted by Lizzette L *et al.,* documented a massive drop in haemoglobin in patients with G6PD deficiency from day 1 to day 7. On day 1 haemoglobin was 12g/dl while on day 7 levels dropped to 6.5 g/dl. Haemolysis leads to increase levels of indirect bilirubin.[12]

Another study conducted by Lopes DV *et al.,* in 2021 reported that methemoglobinemia and haemolytic anaemia are well-documented in G6PD-deficient patients. The mechanism was the same as we discussed in our study by the production of Reactive oxygen species. There was no other trigger for this event except COVID-19.[13] Aguilar *et al.,* in 2020 did the same study and the results were quite similar to ours. Patients until having any other disease or lack of any stimulant remain asymptomatic until triggered by food, drugs, or any other factor.[14]

It is also evident from our study that patients with G6PD deficiency level <1 have Hb below 8g/dl. While patients with deficiency levels of two or three have Hb between 10-12 g/dl, individuals with level 3 deficiency have Hb of 10-16 g/dl. G6PD helps RBCs to maintain their shape and morphology and prevent their lysing. Due to deficiency of G6PD, RBCs become fragile and easy to modify. A 2019 study by Cusimano *et al.,*[15] about the morphology of RBCs in G6PD deficient patients revealed RBCs diameter, the presence of Heinz bodies, and bite cells.

Glader B *et al,* demonstrated that acute haemolytic anaemia is a serious complication of G6PD deficiency. After exposure to a trigger, within no time, in a person with previously normal blood counts, up to two third of cells are rapidly destroyed by oxidative damage. The trigger can be any chemical, drug, bacterial, or viral infection that induces the production of reactive oxygen species. If the acute hemolytic reaction is severe then the prompt treatment is blood transfusions. But if the reaction is less severe then the patient can be resuscitated with fluids and analgesia may be needed.[17]

Simple and accurate tests are required for the diagnosis of G6PD deficiency. The gold standard test is spectrophotometry. It indicates the formation of NADPH from NADP. But this test is expensive and not easily available.[18] G6PD deficiency is an inherited condition with more than 140 genetic variants. Identification of mutations that cause some pathology is important to understand the nature of the genetic disease.

Point mutations in the gene encoding the G6PD enzyme affect the enzyme structure and function by changing the amino acid features and interactions. The polymerase chain reaction test is used to detect special mutations. It is used for screening populations, family studies, and prenatal diagnosis. We conducted this study in AFIP Rawalpindi and the only available PCR detects the Mediterranean variant. The most prevalent mutations of G6PD in Pakistan are the G6PD Mediterranean variant (563C-T), G6PD Chatham (1003A-G), G6PD Orissa (131C-G), and G6PD Karachi.[11] Among these, the G6PD Mediterranean variant is the most common (80%) in the Pakistani population.[11] In our study, Out of 120 individuals, PCR for the Mediterranean variant was positive in 14 individuals who have enzyme activity less than 1 and individuals with G6PD deficiency levels 2-3, 10 out of 120 have positive PCR results.

In a study conducted by Moiz *et al.*,[19] observed different mutations out of which G6PD Mediterranean was most common and dominant. Apart from this, G6PD Chatham and G6PD Orissa were less common. G6PD is located at exon 6 with a single amino acid substitution. G6PD Mediterranean variant was reported in 78% of Pakistanis whereas Chatham and Orissa were less common.[11] Treatment and management mainly depend on the symptoms or complications. Avoidance of causative factors is more important. Anaemia can be treated depending on its severity.

Conclusion

GDPD deficiency can be diagnosed by blood analysis comprising of complete blood count and RBC morphology aided by clinical correlation. The signs and symptoms increase in severity with a decline in GDPD enzyme function along with blood haemoglobin levels. We recommend further studies on large populations.

Limitation: This study was conducted at the Armed Force Institute of Pathology, Rawalpindi. It had certain limitations. First of all only patients who attended and screened in AFIP Rawalpindi are included in the study. It can be much more fruitful if large populations from different parts of the country were screened for G6PD deficiency. Secondly, the time duration was short and this study was hospital-based so such an approach is not that much reliable. Thirdly, the only available

PCR was the one that detects Mediterranean variants, so we were unable to explain further variants in our study.

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