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Determination of Optimal Cut-Off Values of Glucose-6-Phosphate Dehydrogenase Deficiency in a Tertiary Care Hospital

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Abstract

Objective: To define mean cut-off values of glucose-6-phosphate enzyme activity.
Methodology: This cross-sectional study was conducted in the Hematology Laboratory of Shifa International Hospital. Individuals diagnosed with suspected or confirmed G6PD deficiency based on clinical presentation and/or laboratory tests were included. G6PD enzyme activity was quantitatively assessed in whole blood samples using SD Biosensor STANDARD G6PD kits. ROC curve analysis is used to establish the G6PD enzyme cut-off levels.
Results: A total of 120 participants were enrolled in this study. The study included newborns (aged 0-1 month) comprising 1 individual (0.8%), infants (aged 1 month to 1 year) containing 2 individuals (1.7%), pediatric age group (aged 1 year to 12 years) comprising 44 individuals (36.7%), and adults (aged >13 years) comprising 73 individuals (60.8%). Among the male population, 4.30 U/g Hb was established as the cut-off value between normal and deficient individuals. G6PD activity levels above this threshold indicate normal enzyme activity, which was observed in 77.6% of the population. Individuals with G6PD activity below 4.30 U/g Hb were classified as deficient, accounting for 22.4% of the study population. In contrast, the female population was categorized using two distinct cut-off values: 6.50 U/g Hb and 5.45 U/g Hb. Females with G6PD activity above 6.50 U/g Hb were considered to have normal enzyme activity, representing 80.0% of the population. Those with enzyme activity between 5.45 and 6.50 U/g Hb were classified as intermediate, comprising 17.1% of the population. Finally, females with G6PD activity below 5.45 U/g Hb were categorized as deficient, making up 2.9% of the female population.
Conclusion: It is crucial to establish the expected values of G6PD enzyme levels for any population to make a definitive diagnosis. In this study, G6PD enzyme cut-off values were determined for our local population.
Key words: Glucose-6-phosphate dehydrogenase, G6PD Deficiency, G6PD, Enzyme Assays, Cut-off values.

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Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency, an X-linked recessive inborn error of metabolism, is the most prevalent enzyme deficiency, predominantly impacting males. Heterozygous females may display normal, intermediate, or deficient G6PD activity due to the stochastic inactivation of one X chromosome. While G6PD-deficient children typically remain asymptomatic, exposure to specific foods, medications, and certain infectious agents may precipitate hemolytic episodes. Timely detection facilitates early diagnosis by healthcare practitioners, enabling prompt management from an early age. This, in turn, mitigates the potential complications associated with G6PD deficiency, such as acute hemolytic reactions.[1]

For over 30 years, G6PD deficiency testing has been conducted in various countries. An unknown value of G6PD in blood infections can be life-threatening. Therefore, there is a need to set an optimal cutoff value for G6PD enzyme levels. Different optimal cutoff values have been calculated according to country or region. Cutoff values may vary according to gender, age, and regional area.[2]

The established cutoff values for the Chinese population are 2.91 ± 2.77 U/g Hb for males and 6.79 ± 2.61 U/g Hb for females.[3] In contrast, studies conducted in Taiwan reveal a cutoff value of 0.9 ± 0.5 U/g Hb for males and 6.0 ± 2.7 U/g Hb for females.[4] Additionally, a study in Thailand indicates that G6PD- deficient males exhibit a significantly lower value (0.68 ±0.62 U/g Hb) compared to G6PD-deficient females (3.17 ± 1.24 U/g Hb). [5] An optimal level for G6PD also needs to be set for Pakistani population.

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There are diagnostic tests available to identify G6PD deficiency, including quantitative diagnostic tests such as the determination of enzyme activity level using the SD Biosensor STANDARD G6PD test. Based on reflectometry assays, this assay is intended to quantify the concentration of total hemoglobin (g/dL) and G6PD enzymatic activity (U/g Hb) in fresh human whole blood. [6] One significant risk factor associated with G6PD deficiency is the occurrence of severe hyperbilirubinemia, which in turn increases the likelihood of bilirubin neurotoxicity.

Different countries and ethnic groups within a country may exhibit varying mutation spectra and prevalence rates.[7] G6PD value has a wide array of determinants, including geographical distribution of a population, genetics, demography, operating conditions, and criteria for selection, subject preparation and sample collection. Therefore, every population should have its value for G6PD. The objective of this research is to establish cutoff values for G6PD activity, thereby differentiating between individuals with typical, heterozygous, and deficient G6PD levels within the Pakistani population. The SD Biosensor kit for the standard G6PD test is be employed for this purpose.

Methodology

This is a cross-sectional study conducted in the hematology laboratory of Shifa International Hospital (SIH), Pakistan over the period of one year from November 2022 till November 2023. SIH is a 550-bed quaternary care hospital, accredited by the Joint Commission International (JCI) and regularly participates in external quality assurance (EQA) activities conducted by the College of American Pathologists (CAP). On average, the department of hematology receives approximately +-50 blood samples for G6PD analysis per year.

Data was collected for one year after approval of synopsis from the ethical review committee of Shifa International Hospital, Islamabad. All ethical considerations were followed. Written informed consent was taken from all the participants. A predesigned questionnaire with the patient's medical registration number, demographics, family history, and recent drug history was completed. Blood samples were collected in EDTA tubes using standard venipuncture procedures. G6PD enzyme activity was measured using a commercially available spectrophotometric assay kit SD biosensor STANDARD G6PD kit.

A sample size of 120 was calculated by WHO calculator, with confidence interval of 95%, taking absolute precision of I anticipated population mean 6 with SD ± 2.7. Samples were received in EDTA tube. SD Biosensor STANDARD G6PD kits were used for the quantitative measurement of G6PD enzyme activity in a human fresh whole blood sample. Complete blood counts were run on sysmex analyser. Results were recorded as deficient, intermediate or normal in females and normal or deficient in males.

SPSS version 23 was used to analyze the frequency distribution of G6PD values among both affected and unaffected individuals, as well as to perform ROC curve and statistical analyses. The highest attainable value of Youden's J index, calculated at every point on the ROC curve, was used as a criterion for determining the most suitable threshold for classifying G6PD deficiency status.

A p-value of less than 0.05 was deemed statistically significant. The mean ± standard deviation (SD) was computed for the variables Age and G6PD. Gender, normal, heterozygous, and defective people were analyzed to determine their frequency and percentage

Patients were recruited for this study based on the inclusion and exclusion criteria given below.

Inclusion Criteria: Participants of all age groups (neonates, children, adults, and elderly) presenting to Shifa International hospital for G6PD testing. Individuals diagnosed with suspected or confirmed G6PD deficiency based on clinical presentation and/or laboratory tests. Willingness to provide informed consent (or assent for minors) to participate in the study. Patients presenting with symptoms suggestive of G6PD deficiency, such as hemolytic anemia, jaundice, or history of hemolysis after drug intake or infection. Patients admitted during the study period and willing to participate in data collection and testing procedures.

Exclusion Criteria: Individuals without a confirmed or suspected diagnosis of G6PD deficiency. Participants with hemolytic anemia due to causes other than G6PD deficiency (e.g., sickle cell disease, thalassemia). Individuals who have received a blood transfusion within the past 4 weeks should be excluded, as this may

interfere with G6PD enzyme activity measurement. Patients with ongoing hemolysis may experience false normal levels of the G6PD enzyme. Pregnant or lactating women, due to potential risks to the fetus or infant and to avoid confounding results related to hormonal changes. Participants who are unable or unwilling to adhere to study procedures or who fail to comply with follow-up protocols.

Results

A total of 120 participants have been enlisted for the study of G6PD enzyme activity levels. G6PD analysis was conducted on individuals suspected of having G6PD deficiency, as well as those who were not. The average age was calculated to be 20.07 years with a standard deviation of ±18 years. The oldest person was 86 years old, while the youngest was 0.5 years old. The study included newborns (aged 0-1 month) comprising 1 individual (0.8%), infants (aged 1 month to 1 year) containing 2 individuals (1.7%), pediatric age group (aged 1 year to 12 years) comprising 44 individuals (36.7%), and adults (aged >13 years) comprising 73 individuals (60.8%).

There was a higher proportion of males (85, 70.8%) compared to females (35, 29.2%). This research encompasses samples from diverse locations across Pakistan, with the predominant share collected from Islamabad, precisely 88 samples (73.3%). Among the 120 samples, 94 (78.3%) exhibit a normal G6PD enzyme value, while 20 (16.7%) are identified as deficient, and 6 (5%) fall into the intermediate category, as classified by the WHO, as depicted in Table I.

Table I: Frequency and percentage of G6PD Category. (n=120)

G6PD Category	No.	%
Normal	94	78.3
Intermediate	6	5
Deficient	20	16.7

The average Residual Enzyme Activity of G6PD for the entire study cohort is 8.85 ± 4.56 U/g Hb. The average hemoglobin level registers at 11.43 g/dl, with a standard deviation of 3.05 g/dl. The average reticulocyte count is 1.81%, with a range from a minimum of 0.1% to a maximum of 15%.

Among the total males examined, 66 individuals (77.6%) exhibit a normal G6PD enzyme value, while 19 individuals (22.4%) are determined to be deficient. Summarized in Table II, the analysis reveals that out of the total number of females, 28 individuals (80%) are classified as normal, 6 individuals (17.1%) are classified as intermediate, and one individual (2.9%) is classified as deficient in G6PD enzyme.

Table II: Frequency and percentage of G6PD Category with respect to Gender. (n=120)

G6PD Category	Male		Female	
	No.	%	No.	%
Normal	66	77.6	28	80
Intermediate	0	0	6	17.1
Deficient	19	22.4	1	2.9

To establish cutoff values allowing the distinction between intermediate and normal subjects, and between deficient and intermediate subjects, two methods are employed. The first one relies on different G6PD levels established according to the WHO classification. The second approach involves ROC curves analysis. For the overall population, the ROC analysis determines a cutoff at 6.05 U/g Hb for normal G6PD, achieving a specificity and sensitivity of 100% illustrated in Figure 1.

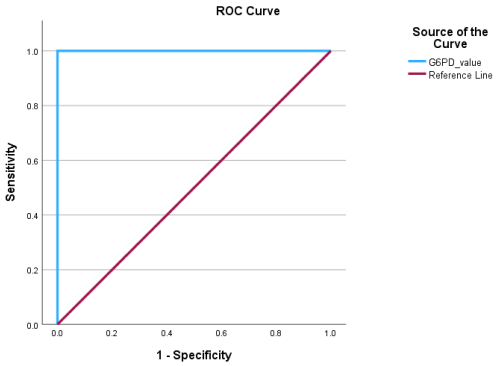

Figure I: ROC curve for Total Population (n=120)

Gini Index	K-S Statistics	
	Max K-S[a]	Cut-off
1.000	1.000	6.0500

In the male population, a cutoff of 4.30 U/g Hb is established through ROC curve analysis, also yielding a specificity and sensitivity of 100%. This results in an effective discrimination between deficient and normal males. In the female group, there exist categories of normal, intermediate, and deficient. ROC cutoff values of 6.50 U/g Hb and 5.45 U/g Hb are established to discriminate between normal, intermediate, and deficient women shown in figure 2 and 3 respectively. Individuals with G6PD deficiency exhibited accompanying characteristics such as a positive family history, clinical manifestations like jaundice, or elevated hemolytic

markers. The peripheral film analysis of red blood cell morphology revealed bite cells, nucleated RBCs, spherocytes, Heinz bodies, polychromasia, and blister cells, providing additional confirmation of the result.

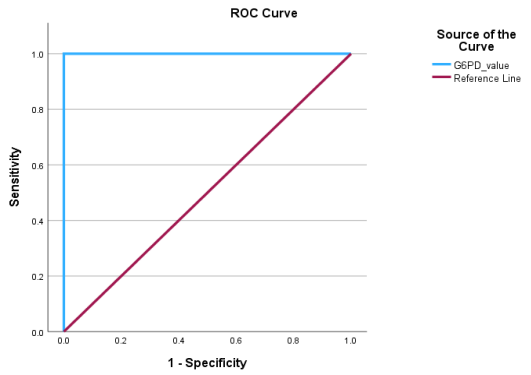

Figure 2. ROC curve for Male Population. (n=85)

Gini Index	K-S Statistics	
	Max K-S[a]	Cut-off
1.000	1.000	4.3000

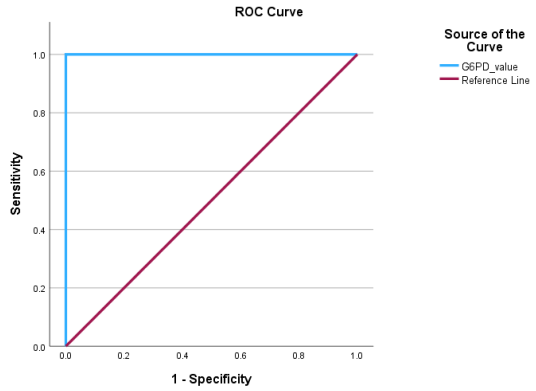

Figure 3. ROC curve for Female Population. (n=35)

Gini Index	K-S Statistics	
	Max K-S[a]	Cut-off
1.000	1.000	5.45
1.000	1.000	5.45

Discussion

G6PD deficiency, recognized as the most prevalent enzyme-related disorder globally, is estimated to impact around 400 million people. [8] This deficiency exhibits varying prevalence rates across regions, with higher incidence reported in Africa, Southern Europe, the Middle East, Southeast Asia, and the central and southern Pacific Islands. [9] Notably, a recent meta-analysis suggested a global prevalence of 4.5%, yet local findings in Pakistan, particularly among Pathans, contradicted this, revealing a higher incidence of 8%. Discrepancies in estimates highlight the need for comprehensive, region-specific studies.

In Pakistan, neonatal jaundice is a significant concern, accounting for a substantial portion of hospital admissions. Various factors contribute to jaundice, with G6PD deficiency identified in 8% of affected infants. [10] Further studies in Afghan refugees and the KPK Province of Pakistan revealed varying prevalence rates among different communities, with Pathan refugees exhibiting a noteworthy 15.8%. Additionally, hospital studies emphasized the life-threatening hemolytic crises associated with the specific form of G6PD deficiency in Pathans. [11] The implications of G6PD deficiency extend to malaria treatment strategies, particularly in Pathan communities where primaquine use poses severe risks. The study suggests the necessity of revising recommendations for primaquine use in regions with high G6PD deficiency prevalence, underscoring the importance of tailoring interventions to local contexts. [12]

Primaquine can only be used in individuals with G6PD activity above 30%. People whose enzymatic activity is less than 30% shouldn't be given the 14-day primaquine regimen. Prescribers should be aware of the possibility of hemolysis in patients with moderate G6PD insufficiency (30% to < 70% activity), since clinically meaningful hemolysis can still occur in these patients. If hemolysis develops, primaquine should be stopped immediately. [13]

Genetic background affects the cut-off values of G6PD activity to identify G6PD deficiency since the G6PD gene is highly polymorphic with numerous variations, which may result in G6PD deficiency or lower G6PD activity in erythrocytes. [14] For example, a heterozygote with 80% G6PD deficient red blood cells (mean residual activity of the G6PD A− allele 18%) is likely to have more brisk AHA after being exposed to primaquine than a heterozygote with 20% G6PD deficient red blood cells (mean residual activity of the Mediterranean allele 4.4%). [15]

Quantification methods for G6PD deficiency play a crucial role in accurately estimating prevalence. Qualitative techniques, such as Formazan ring tests, fluorescence spot tests, and others are beneficial for mass screening but may miss cases of partial deficiency, especially in females. [16] A recent study suggests the effectiveness of a qualitative test with a 30% cutoff in distinguishing between males with deficient and normal

levels. However, these tests exhibit lower efficiency in females. [17]

To address these limitations, our study focused on establishing cutoff values for G6PD screening, mainly targeting increased sensitivity in detecting female heterozygotes. For male subjects, the conventional cutoff value of 4.3 U/g Hb effectively distinguishes between normal and deficient subjects. [18] Importantly, for the female population, our study established a reliable cutoff value to distinguish heterozygous females from those with normal or deficient levels. Notably, the prevalence of G6PD deficiency in females accounted for 2.9% of the overall study population. The female patient who was classified as deficient exhibited G6PD activity below the established threshold of 5.45 U/g Hb, supporting the validity of the cut-off values used in this study. The lower prevalence of G6PD deficiency in females, compared to males, is attributed to the X-linked inheritance pattern of the disorder. Since females have two X chromosomes, a defective gene on one X chromosome is often compensated by the normal allele on the other X. This heterozygous advantage can result in females with milder forms of G6PD deficiency, which may be underdiagnosed or less severe in their clinical presentation.

Furthermore, the female subject identified as deficient might represent a homozygous individual or one with significant skewed X-inactivation, leading to a more pronounced deficiency. The cut-off values established for females in this study, 6.50 U/g Hb for normal, 5.45 U/g Hb for deficient were shown to accurately differentiate between the normal and deficient categories, with the solitary case of a deficient female supporting the robustness of the thresholds. These defined cutoffs offer the advantages of cost savings and quicker turnaround time, eliminating the need for additional genetic analysis, as validated by positive family history and clinical presentation in deficient cases. [19] Although a complementary information on underlying G6PD variations and heterozygosity is provided by further procedures including flow cytometry and genotyping. The majority of test assessments have employed spectrophotometry as the reference method. [20]

To the best of our knowledge, no earlier research in Islamabad revealed precise cutoff values for G6PD. When this study was completed, the cut-off values obtained were different from the ones set by the manufacturer. Therefore, our findings provide a clear method to help with G6PD deficiency screening, increasing screening accuracy, lowering needless worry from false positives, and conserving medical resources.

Conclusion

This study emphasizes the importance of accurate diagnostic methods for G6PD deficiency, underscoring the need for normative G6PD enzyme levels to facilitate a definitive diagnosis. It addresses the lack of region-specific data, particularly in Pakistan, where G6PD prevalence varies, and underscores the necessity for tailored reference values that reflect the local genetic and environmental context. The inclusion of ROC curve analysis, in conjunction with DNA testing, enhances diagnostic precision and offers an alternative method for determining G6PD status. The study recommends targeted research on region-specific normative values and highlights the potential of ROC curve analysis in enhancing diagnostic accuracy. Ultimately, the findings contribute to the advancement of precision medicine and personalized healthcare, advocating for tailored diagnostic strategies in diverse populations.

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