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Original Article

Diagnostic Accuracy of G6PD Biosensor Analyzer for the Screening of Glucose-6-Phosphate Dehydrogenase Deficiency

Khadija Iftikhar, Chaudhry Altaf, Rafia Mahmood, Ayesha Khurshid, Ali Ahmed, Saqib Hussain Korejo
Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi.

Abstract

Background: Glucose 6 phosphate dehydrogenase (G6PD) deficiency is an inherited enzymopathy that is associated with hemolytic anemia.
Objective: To evaluate the diagnostic accuracy of G6PD biosensor analyzer used at study site for the quantitative measurement of total G6PD enzyme activity in whole blood using Trinity biotech quantitative assay as gold standard.
Study type, settings and duration: This cross-sectional study was conducted in the Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi from April 2018 to September 2018.
Methodology: One hundred and thirty seven patients having unexplained hemolytic anemia and suspected to have G6PD deficiency were included in the study using non-probability consecutive sampling technique. Detailed history, including drug history and transfusion history was taken and a complete physical examination was done. Each eligible patient was analyzed by G6PD biosensor analyzer for the quantitative measurement of total G6PD enzyme activity while Trinity biotech quantitative assay was used as gold standard.
Results: A total of 137 individuals with unexplained haemolytic anaemia suspected of having G6PD deficiency were enrolled in our study. On statistical analysis, G6PD Biosensor Analyzer was found to be 96.08% (95% CI: 86.54-99.52%) sensitive and 89.53% (95% CI:81.06-95.10%) specific as compared to our gold standard method Trinity Biotech. The positive likelihood ratio was 9.18 (95% CI:4.94-17.08) while the negative likelihood ratio was 0.04 (95% CI:0.01-0.17) with a positive predictive value and a negative predictive value of 84.48% (95% CI: 74.53-91.01%) and 97.47% (95% CI:90.81-99.34%), respectively. The accuracy of our test determined to be 91.97% (95% CI:86.09-95.92%).
Conclusion: G6PD Biosensor Analyzer is a rapid and cost-effective method for diagnosis of G6PD deficiency.
Key words: G6PD deficiency, diagnostic accuracy, G6PD quantitative assay.

Introduction

Glucose 6 Phosphate Dehydrogenase (G6PD) deficiency is an inborn error of metabolism with a predisposition to red cell breakdown.[1] G6PD deficiency, the most common of the inherited enzyme deficiencies, is an X-linked disease, predominantly seen in males.[2] The gene for G6PD is located on the X chromosome (Xq28).[3] G6PD deficiency, though seen worldwide but this disorder has gained special attention in malaria endemic areas as patients deficient in G6PD have a possible protection against malaria.[4] This also correlates with higher prevalence of G6PD deficiency in areas where malaria is more prevalent.[5]

The role of glucose-6-phosphate dehydrogenase in red cell metabolism is of prime importance.[1] The enzyme, G6PD, catalyzes the first step of the hexose monophosphate shunt (HMP), and is the enzyme controlling flux through the pathway, rather, they are transferred from nicotinamide adenine dinucleotide phosphate (NADPH) to a tightly bound flavin adenine dinucleotide (FAD) on the reductase, then to a disulfide bridge between two cysteine residues in the enzyme subunit, and finally to oxidized

Corresponding Author:
Khadija Iftikhar
Department of Haematology
Armed Forces Institute of Pathology
CMH Road, Rawalpindi.
Email: khadijashahzeb@gmail.com

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Authors Contribution

KI & CA conceptualized the project. KI & RM did the data collection. KI, CA, RM & AA did the literature search. KI, RM, AK & SHK performed the statistical analysis. Drafting, revision and writing of manuscript was also done by KI, RM & AK.

glutathione.[6] The hexose monophosphate shunt, thus, protects the red cells against oxidative injury by producing NADPH, a co-factor required in glutathione metabolism.[7,8] Therefore, G6PD deficiency leads to decreased NADPH production, leading to decreased reduced glutathione (GSH), ultimately making the cell susceptible to oxidative injury leading to red cell haemolysis.[9] Haemolysis is mainly extravascular but some degree of intravascular haemolysis also occurs.[6] Haemolysis results in the clinical signs of anaemia and jaundice.[2]

G6PD deficiency presents as a spectrum of disorders with some patients being asymptomatic for years, their symptoms precipitating with intake of medications; while others may present very early with neonatal jaundice.[9,10] Other clinical presentations include chronic non-spherocytic haemolytic anaemia and acute exacerbation of haemolytic anaemia by ingestion of fava beans.[11] Diagnosis of G6PD deficiency may however, be challenging as not only the severity of anaemia and clinical presentations are variable among individuals with G6PD deficiency, but also there are a large number of inciting factors leading to haemolysis.[12] Clinical suspicion and unexplained haemolytic anaemia warrants work-up to reach a diagnosis. Typically patients insidiously develop anemic symptoms such as weakness, dizziness, fatigue and dyspnea on exertion; other less specific symptoms include fever, bleeding, coughing, abdominal pain and weight loss. CBC, peripheral film examination, Heinz body preparation and testing of urine for haemoglobinuria and haemosiderinuria are preliminary investigations carried out.[13,14] Diagnostic evaluation then includes screening tests followed by confirmatory tests, or directly confirmatory tests depending not only on the protocol being followed but also on the available resources and cost constraints.[15] Molecular testing is also available, though mostly used for research purposes.[16] The importance of early diagnosis cannot be overemphasized. Timely diagnosis allows education of the patient regarding precipitating factors and events, safe and unsafe foods and medications, thus preventing haemolytic episodes.[17]

Developing countries like Pakistan have limited resources available for confirmatory testing and the diagnosis of many patients with unexplained haemolyticanaemia remains unidentified. Epidemiologically, Pakistan is classified as a moderate malaria endemic country and G6PD deficiency is common in Pakistan, though, definitely under-reported as many patients remain undiagnosed. The G6PD biosensor is a quantitative point of care test based on the electrochemical principle of G6PD enzyme activity by measuring the electricity generated by reduction of NADPH coupled with the ferricyanide.[18] The rationale of our study was to evaluate the diagnostic accuracy of G6PD biosensor analyzer for the quantitative measurement of total G6PD enzyme activity in whole blood using an electrochemical method. This is a cost effective method and can provide rapid diagnosis. This will help identification of patients with G6PD deficiency, thus allowing better management; earlier institution of preventive strategies; education regarding triggering factors, avoidance of unsafe drugs and dietary restrictions to prevent acute exacerbations; and genetic counseling of parents and advice regarding importance of early neonatal screening.

Methodology

This cross-sectional study was conducted in the Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi, from April 2018 to September 2018. Patients of both genders and all ages, from neonates to adults, were included in the study with an age range of 0-76 years. All subjects were elaborately apprised about the study and written informed consent was obtained from them. For the patients below the age of 15 years written consent was taken from the guardian. All patients having unexplained haemolytic anaemia and suspected to have G6PD deficiency were included in the study. While patients with high reticulocyte count were excluded so as to avoid false negative results.

Detailed history including drug history and transfusion history along with complete physical examination of each study was done. Symptoms and signs were noted. Participants blood tests i.e. CBC, peripheral blood film and reticulocyte were done. Coombs test (urine for haemosiderin), bilirubin levels and serum LDH were done. Urine was tested for haemoglobin. Heinz body preparation was made and examined. Screening tests i.e. methaemoglobin reduction test and dye reduction test were performed on all samples. Abdominal ultrasound was done for splenomegaly or any other complications.

The G6PD Biosensor analyzer based on the electrochemical principle of G6PD enzyme activity by measuring the electricity generated by reduction of NADPH coupled with ferricyanide was the index test. A 2ml EDTA blood sample was collected from each patient and 10µl sample was added to extraction buffer cuvette provided by the manufacturer. It was then mixed and allowed to settle for 10 minutes. 10µl of this sample was then drawn in a pipette and applied to specimen application hole of the test device. Test result appeared on the screen after 2 minutes. Cut-off value <2u/g Hb (which equates to <30% of adjusted median G6PD enzyme activity based on manufacturer's instructions) was kept as diagnostic

of G6PD deficiency. Trinity Biotech Quantitative Assay was used as gold standard test. The enzyme activity was determined by measuring the change in absorbance at a wavelength of 340nm after a 05 minute incubation at room temperature and a cutoff value <4.6 u/g Hb was diagnostic of deficiency (as per manufacturer's instructions).

Collected data was entered and analyzed using SPSS version 24. Descriptive analyses was carried out after confirming the Gaussian nature of the data by test of normalcy. Quantitative variables were presented by mean and SD while qualitative variables were presented by frequency and percentage.

Ethical clearance was taken from Ethical Review Committee of Armed Forces Institute of Pathology, Rawalpindi.

Results

Sample size was estimated using Open epi sample size calculator, assuming proportion of population as 50%, margin of error as 5% and 95% confidence level. A total of 137 individuals with unexplained haemolytic anaemia suspected of having G6PD deficiency were enrolled in this study with the mean age of 18.5±17.2 years (Figure-1). Of these, 111 (81%) were males while 26 (19%) were females. The patient population comprised of all ages from newborns to adults to elderly, with an age range of 0-76 years. Among them 62 (45.3%) patients were in the paediatric age group (0-18 years) while 70 (51.1%) were adults (19-60 years) and 5 (3.6%) were elderly patients (61-76 years) (Figure-1).

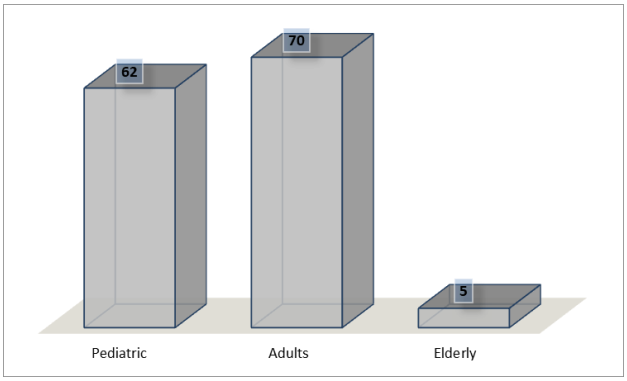

Figure: Frequency distribution of age.

These patients were then divided into two groups based on presenting clinical features. Group A comprised of individuals with anaemia and neonatal jaundice while group B had individuals with anaemia, jaundice and/or splenomegaly or drug allergy. On stratification into groups based on the presenting clinical features, 117 (85.4%) were in group A while 20 (14.6%) were in group B (Table-1).

Table 1: Descriptive statistics of the study population.

Variables	*n (%)*
Gender	
Male	111 (81)
Female	26 (19)
Clinical presentation	
Group A	117 (85.4)
Group B	20 (14.6)

On quantitative analysis using the gold standard Quantitative Assay, 51 (37.2%) patients were deficient while 86 (62.8%) had G6PD levels more than our cut-off value of 4.6 U/g Hb. However, on testing for G6PD levels by our index test using G6PD Biosensor Analyzer, 58 (42.3%) patients were found to be deficient using cut-off value of 2U/g Hb while 79 (57.7%) patients did not show G6PD deficiency having enzyme levels above the designated threshold. On statistical analysis, G6PD Biosensor analyzer was found to be 96.08% (95% CI:86.54-99.52%) sensitive and 89.53% (95% CI:81.06-95.10%) specific as compared to the gold standard (G6PDH quantitative) assay. The positive likelihood ratio was 9.18 (95% CI:4.94-17.08) while the negative likelihood ratio was 0.04 (95% CI:0.01-0.17) with a positive predictive value and a negative predictive values of 84.48% (95% CI:74.53-91.01%) and 97.47% (95% CI:90.81-99.34%), respectively. The accuracy of our test determined to be 91.97% (95% CI:86.09-95.92%) (Table-2).

Table 2: Biosensor assay results versus trinity biotech results.

Biosensor Assay	*Trinity Biotech Results*		
	Deficient n (%)	*Normal n (%)*	*Total n (%)*
Deficient	49 (96.1)	9 (10.5%)	58 (42.3)
Normal	2 (3.9)	77 (89.5	79 (57.7)
Total	51 (100)	86 (100)	137 (100)

Discussion

G6PD deficiency is the commonest enzymopathy worldwide. Considering the endemicity of malaria in our region and its association with G6PD deficiency, G6PD deficiency is not uncommon in our region, though, definitely under-reported and for that reason the diagnosis of G6PD deficiency becomes pivotal in our setting. The exact incidence of the disease has not been established in our population. In developing countries like Pakistan, G6PD deficiency remains a diagnostic dilemma.

While molecular analysis is definitive, however, in under-resourced countries with limited access to healthcare facilities, most patients with G6PD deficiency remain undiagnosed. Early diagnosis of G6PD deficiency is of utmost importance in helping these individuals in striving to live a healthy life.

In this study, we have evaluated the quantitative point of care G6PD biosensor analyzer for the assessment of G6PD activity in our patients. We have found G6PD biosensor analyzer to have a sensitivity of 96.08% and a specificity of 89.53% with an accuracy of 91.97%. Another study conducted by Banconein Thailand,[19] G6PD biosensor analyzer was found to be 100% sensitive and 92% specific at <30% G6PD spectrophotometric activity. This study, however, reports a sensitivity of 92% and a specificity of 94% in samples with <80% G6PD activity. In our study, G6PD biosensor analyzer serves as an excellent point of care test having a high negative predictive value of 97.47%, thus, truly ruling out disease. Von Fricken [20] screened 456 participants in Gressier, Haiti using G6PD biosensor analyzer. He reported a negative predictive value of 98.2% which is in accordance with our findings. Thus, G6PD biosensor analyzer is an effective and efficient tool for the early diagnosis of G6PD deficiency. This is not only cost-effective but also has a very short turnaround time and can be extremely helpful in prompt diagnosis, correct management and early institution of preventive measures. However, studies on larger scales need to be considered in the future and comparison of these methods with molecular diagnosis may prove useful.

Conflict of interest: None declared.

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