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Original Research Article

Comparative Analysis of Qualitative G6PDH Assay with Gold Standard Quantitative Assay to Detect G6PD Deficiency in Pediatric Patients

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Abstract

Background: G6PD is the rate limiting enzyme in the pentose phosphate pathway that protects human cells from oxidative stress. G6PD deficiency is one of the most common enzymopathies in humans, affecting an estimated 400 million individuals worldwide. The main objective of our study is to Compare diagnostic accuracy of screening qualitative G6PDH assay with standard quantitative assay. ***Methodology and Results:*** This is 2 years study comprising 250 confirmed cases of G6PD deficiency by UDILIPSE G6PD quantitative assay. Of which 210 were boys and 40 were girls. 40 cases were reported as false Normal by G6PDH screening assay in boys and 28 in girls. ***Discussion:*** Our results were comparable to studies conducted by Mohammed islam, Daae LN *et al.*, Bancone G *et al.*, Kahn M *et al.*, ***Conclusion:*** It can be safely concluded that male patients with suspected G6PD deficiency can be screened for G6PDH assay and if tests are indeterminate then can be proceed to quantitative assay. For female patients it is recommended to omit screening test and can directly perform quantitative G6PDH assay in order not to miss G6PD deficient carriers.

Keywords – G6PD deficiency, Antimalarials, Homozygous.

INTRODUCTION

Glucose 6 Phosphate Dehydrogenase (G6PD) is the rate limiting enzyme in the pentose phosphate pathway that protects human cells from oxidative stress. G6PD generates reduced NADPH which binds with free radicals and protect from oxidative stress [1].

G6PD deficiency is one of the most common enzymopathies in humans, affecting an estimated 400 million individuals worldwide [2]. It is X-linked disorder, males can be either hemizygous normal or hemizygous deficient, whereas females may be either homozygous normal, homozygous deficient or heterozygous. A heterozygous female will be a mosaic for cells expressing the wild type enzyme and cells expressing a deficient variant. The variable proportion of normal and deficient red cells, as a consequence of random X-chromosome inactivation (Lyonisation), renders diagnosis in some female heterozygotes difficult [3, 4].

A fraction of red cells in heterozygotes (on average, 50%) is as enzyme-deficient as in hemizygous males and therefore susceptible to haemolysis. The severity of haemolysis and its potential clinical complications are roughly proportional to the fraction of deficient red cells. As a result of the random nature of X-chromosome inactivation during embryogenesis, individual females can manifest skewing in favour of either the normal or deficient G6PD allele [5].

Indications for G6PD testing

- Before Starting Antimalarials and oxidant drugs
- Prolonged or severe neonatal jaundice
- Favism
- Congenital non-spherocytic haemolytic anaemia in males or females
- Haemoglobinuria
- Sickle cell disease
- Thalassaemic disorders
- Family history of G6PD deficiency or favism

To date more than 185 clinical variants of the G6PD gene have been reported, associated with a wide spectrum of enzyme activity [6]. ICSH recommends Quantitative G6PDH assay as the gold standard for detection of G6PD deficiency [7].

OBJECTIVES

1. To Compare diagnostic accuracy of screening qualitative G6PDH assay against standard quantitative assay
2. To determine incidence of G6PD deficiency in boys and girls
3. To study effectiveness of Quantitative G6PDH assays in girls
4. To propose investigative algorithm for G6PD deficiency

METHODOLOGY

This is 2 years study conducted in hematology section, MMCH, KSA. Institutional review committee provided ethical approval and permission. The local policy was followed for obtaining consent

Inclusion Criteria

1. As per the Hospital policy, children upto 14 years were included
2. G6PD deficient boys and girls tested with quantitative assays

Exclusion Criteria

1. Blood Transfused
2. PK deficiency
3. HDN

Principles of measurement of G6PD activity

All tests for measuring G6PD activity depend on detecting the rate of reduction of NADP to NADPH

1. Absorption of light at 340 nm.
2. Fluorescence produced by long wavelength UV light (approximately 340 nm).
3. Ability to decolourise or lead to the precipitation of certain dyes.

Check the absorbance of the spectrophotometer for NADPH at the bandwidth (slot width) used and use the value obtained in your calculations. The molar extinction 6.22 is only obtained with narrow (≤4 nm) bandwidth instruments that are regularly serviced.

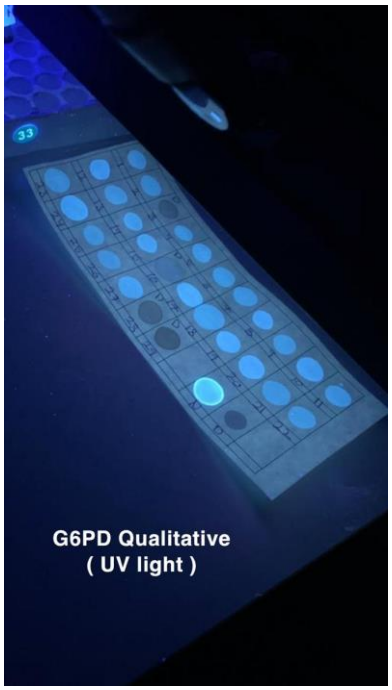
Measure HGB concentration. Normal range for G6PDH assay is 8.7 +/- 1.7 iu/g HGB at 30°C; 12.1 +/- 2.09 iu/g HGB at 37°C (without correction for 6-PGD activity) and 8.34 +/- 1.59 iu/g HGB at 37°C (corrected for 6-PGD activity)

Staining of Blood and BMA smears

Smears were stained withmodified RomanowskyWright- Giemsa stain. BMA smears were Fixed in wright stain for 5 min and stained with Giemsa stain for 10-15 min, washed with distilled water and studied under microscope.

Statistical analysis of data

Mean +/- SD was used to express all data. Utilizing the unpaired students t test, statistical analysis was conducted. Statistically significant data has a p value less than 0.05.



G6PD screening test

UDILIPSE G6PD quantitative assay

RESULTS

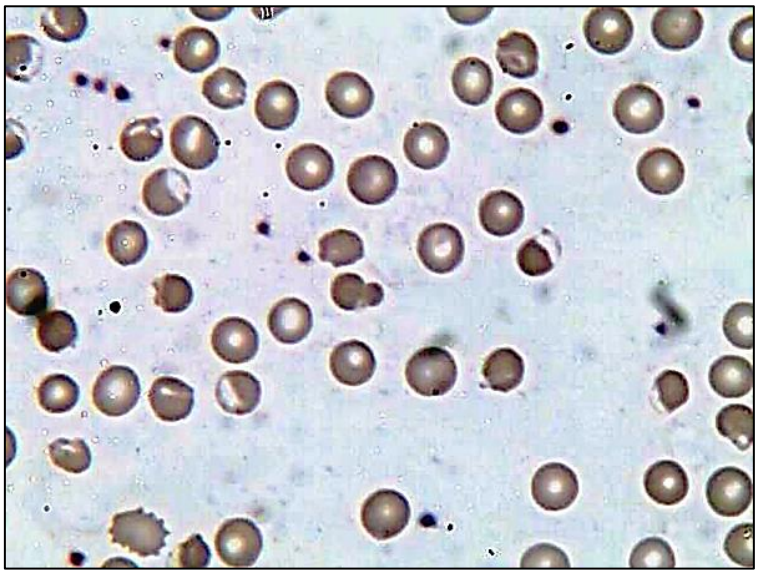
A total of 250 G6PD deficient cases diagnosed by quantitative G6PDH assay were studied. Of which 210 were boys and 40 were girls.

Table – Comparative Analysis of qualitative and quantitative G6PD assay

Gender	Boys	Girls
G6PDH quantitative assay	210	40
G6PD screening assay	170	12

40 cases (19%) were reported as false Normal (missed G6PD detection) in boys and 28(70%) in girls. This high proportion of girls were false normal due to carrier sate. For these childrens with false Normal G6PDH assay peripheral smear shows Bite cells and retic % was increased in all cases.

Based on this findings it is recommended that Hb, Retic% and blood smear should be done mandatorily along with G6PDH screening assay were the facility of quantitative assay is not available, so that G6PD deficient cases are not missed.



Bite cells in a case of G6PD deficiency

The G6PD variants can be classified as Class 1 (<10% residual G6PDH activity) taken as % of normal to class V (100% activity). Class 1 patients usually presents as Chronic non spherocytic hemolytic anemia in neonates manifesting as neonatal jaundice. [1]. Certain Drugs (Antimalarials, Analgesics, Antipyeritcs) triggers hemolysis in G6PD deficient individuals.

ICSH recommends fluorescence screening test for G6PD deficiency by modified Beutler and Mitchell method. This can be confirmed by quantitative enzyme assay, the methaemoglobin reduction test. Red cells with <20% of normal G6PD activity do not cause detectable fluorescence.

The main causes of erroneous interpretations are as follows

False Normal: Reticulocytosis can cause false normal G6PDH assay because young RBCs have more G6PD activity. So it is ideal to test for G6PDH assay when Retic% is normal.

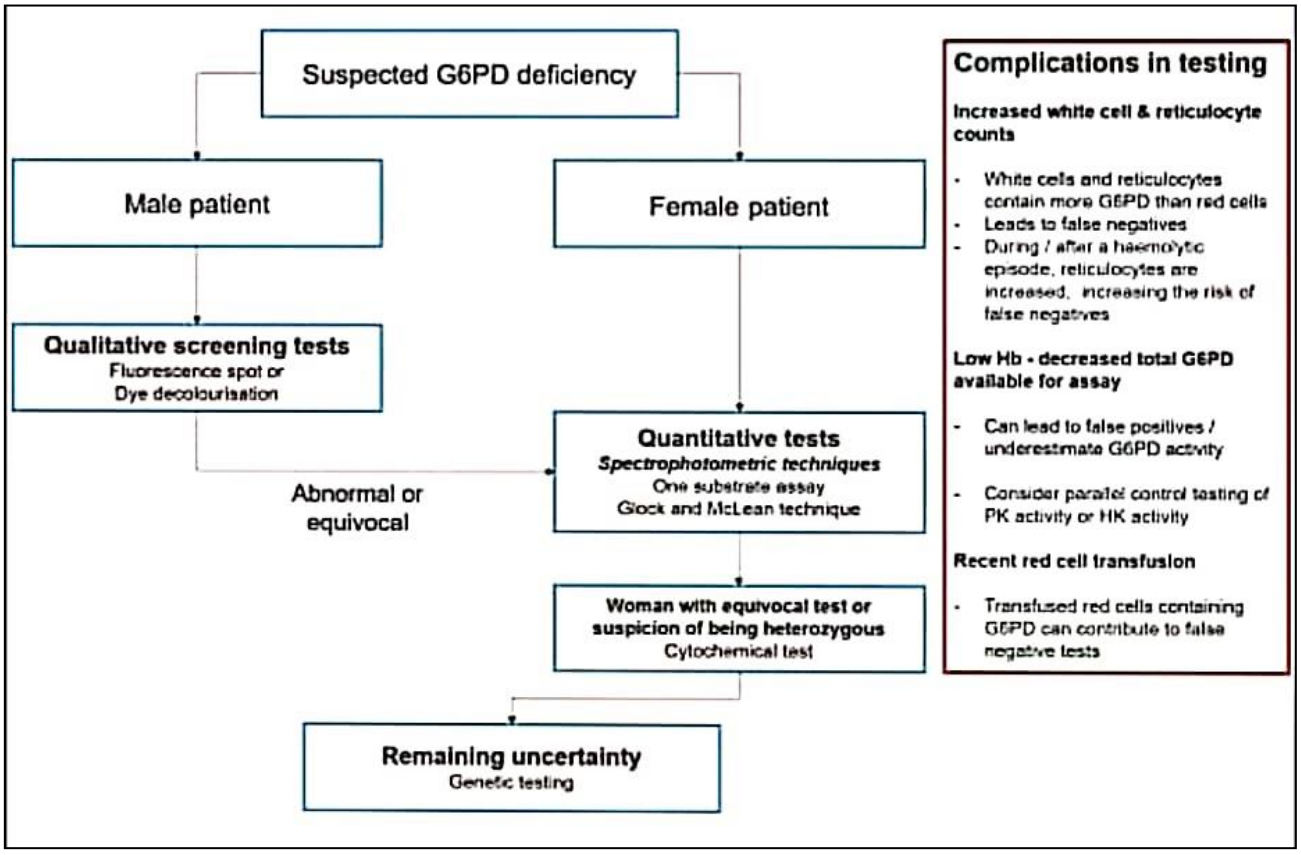
False-Deficient: In anaemic patients, screening tests for G6PD deficiency fail to demonstrate most heterozygotes. The deficient redcells canbe identified in blood films by a cytochemical elution procedure which may be useful in genetic studies and in assessing G6PD activity in women.

Other study by Benedikt Ley, Mohammad Shafiul Alam showed a 9% prevalence of G6PD deficiency, 7.5% (72/965) of patients with a normal Biosensor result were actually severe or moderate deficient (<30% activity), 52.8% (38/72) of those were severe deficient (<10% activity) according to spectrophotometry. Overall only 19% (17/89) of severe or moderate deficient individuals (<30% G6PD activity) would have been identified by the Biosensor [2, 8].

Studies conducted by Daae LN *et al.*, Bancone G *et al.*, Kahn M *et al.*, showed results comparable to present study [9, 10].

Recommendations

1) *If a male patient is suspected of having G6PD deficiency on clinical grounds, either the fluorescent spot or the dye decolourisation screening test are acceptable first-line tests. The complications of a raised white blood cell count, raised retics, low Hb (and the inability to reliably identify female carriers) must be understood. If the screening test is abnormal or equivocal, the quantitative assay should be undertaken to confirm, or exclude, the diagnosis unless the patient is known to the laboratory. It is best to proceed directly to quantitative assay for female patients, who may be heterozygous and thereby possibly misclassified by any screening test.*
2) *If a woman has an intermediate or equivocal result in the quantitative assay then the cytochemical test should be undertaken. If there is a clinical or genetic reason to suspect that a woman is heterozygous for G6PD deficiency then the cytochemical test should be undertaken even if the quantitative assay is normal because the cytochemical test may be the only way to detect a deficiency in some cases (other than by DNA analysis).*
3) *The final G6PD activity should be interpreted in light of the reticulocyte count measured on the same sample.*
4) *The MCH (pg) of the test sample should be taken into account, as very low values (as seen in thalassaemia and iron deficiency) will overestimate the G6PD level where results are expressed in units per g haemoglobin.*
5) *Based on present study findings it is recommended that Hb, Retic% and blood smear should be done mandatorily along with G6PDH screening assay were the facility of quantitative assay is not available, so that G6PD deficient cases are not missed.*



Investigative pathway for G6PD deficiency – Proposed Algorithm

Limitations of study

1) Genetic test was not available
2) Detail history of patients was not available

CONCLUSION

It can be safely concluded that male patients with suspected G6PD deficiency can be screened for G6PDH assay and if tests are indeterminate then can be proceed to quantitative assay. For female patients it is recommended to omit screening test and can directly perform quantitative G6PDH assay in order not to miss G6PD deficient carriers

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