Rate of G6PD deficiency in male population: A comparison between two methods

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Abstract: Background: To compare two methods (formazan ring method and dye decolorization method) of G6PD screening and to determine the prevalence rate of G6PD deficiency in male children aged 0 to 7 years admitted in ward or outpatient department in Pediatric Hospital of Karachi, Pakistan. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most frequent and commonest X-linked red cell enzymopthyies, hemizygous males and homozygous females are the ones that are mainly affected. Deficient subjects become symptomatic when there is oxidative stress induced by infection, drugs etc Approximately 400 million people carry the defective gene globally, and the recent meta-analysis reported prevalence of 4.5%. Various local population based studies have shown that G6PD deficiency is not a rarity in Pakistan, reporting prevalence of 2 to 3.8% with highest frequency of 8.6% observed in Pathans. **Material and Methods:** Hospital based cross-sectional study conducted at National Institute of Child Health, from August' 2014 to November' 2014. 100 samples were collected with a clinical history of jaundice, clinical and biochemical history of anemia. The recruited subjects were screened using a commercially available kit based on Dye Decolorization method which is a qualitative visual colorimetric assay. The frequency of G6PD deficiency was determined using SPSS 16.0. **Results:** Out of hundred males screened, 9 (9%) were found to be G6PD deficient. The mean age was 3.6 years and mean hemoglobin was 6.8 mg/dL. Of the 9 screened positive 5 were clinical jaundiced and 7 were both clinical and biochemically anemic. **Conclusions:** This study like others provide evidence to believe that G6PD deficiency is not a rarity in Pakistan. 9% of prevalence reported in this study, emphasizes on the need to conduct epidemiological studies to determine the carrier rate, whether we need to screen all our neonates and to find out which screening and confirmatory test is cost-effective to be adapted in our setup as a part of screening program.

[Umme-Hani, Zumar Hafeez, Muzammal Mateen Azhar, Aamer Mahmood Qazi and Nida Bai. **Rate of G6PD deficiency in male population: A comparison between two methods.** *N Y Sci J* 2019;12(3):1-3]. ISSN 1554-0200 (print); ISSN 2375-723X (online). http://www.sciencepub.net/newyork. 1. doi:10.7537/marsnys120319.01.

Keywords: Glucose-6-phosphate dehydrogenase, X-linked red cell, hemizygous, oxidative stress, anemic

Introduction

The enzyme glucose-6-phosphate dehydrogenase (G6PD) is essential for RBCs to complete their normal life span and for oxidation process. G6PD also protects red blood cells from potentially harmful byproducts that can accumulate when a person takes certain medications or when the body is fighting an infection. Deficiency of the G6PD enzyme results in sudden destruction of red blood cells due to oxidative damage (1). It is one of the enzymes of the pentose phosphate pathway. This pathway is involved in keeping an adequate amount of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH) in cells. NADPH in turn maintains the levels of glutathione which protects the red cell from oxidative damage. G6PD is the rate-limiting enzyme in the pentose phosphate pathway (2). G6PD deficiency is a hereditary X-linked disorder characterized by abnormality in RBCs and leads to breakdown of RBCs prematurely. This defect is more common in males as it is X-linked defect. This disease causes hemolytic anemia followed by the use of drugs during infections, intake of fava beans, and certain legumes. Glucose-6-dehydrogenase deficiency is also a significant cause of mild to severe jaundice in newborns.

Pathophysiology

Red blood cells are constantly challenged by oxidants in the form of free radicals generated by the conversion of oxyhemoglobin to deoxyhemoglobin and by peroxides generated by phagocytosing granulocytes. Normal red cells can increase generation of NADPH in response to oxidative stress; this capacity is impaired in patients with G6PD deficiency. Failure to withstand oxidative stress damages sulfhydryl groups in hemoglobin and the red cell membrane and causes hemolysis. Cells in other tissues and organs have alternate pathways for the generation of NADPH and can thus withstand such oxidative stress. In contrast, red cells are metabolically extremely simple; they lack a nucleus and mitochondria, cannot carry out protein synthesis, and exclusively metabolize glucose for ATP production. The activity of all red cell enzymes, including G6PD, is highest in young red cells (reticulocytes), and progressively declines as the cell ages.

Frequency

It is estimated to affect 400 million people worldwide with the highest prevalence rates in tropical Africa, the Middle East, tropical and subtropical Asia and some parts of the Mediterranean region. In Asia, there is wide variation in reported incidence of G6PD deficiency across the different countries: 1.2% in Sri Lanka, 0–17.3% in India, 9.8% in Iran, 0.5% in Japan, 3.5-16.7% in Malaysia, 1.5–3.4% in Singapore, 2.8–14.3% in Thailand, 4.5-25.7% in the Philippines, and in Pakistan G6PD deficiency rate is 2%-3%.

Signs and symptoms

Most individuals with G6PD deficiency are asymptomatic, but a child may exhibit symptoms of hemolytic anemia. In some cases, hemolytic anemia is self limiting. In newborn g6pd deficiency causes neonatal jaundice, possibly leading to kernicterus. These symptoms usually disappear when the offending food or drug is stopped (3).

Diagnosing G6PD deficiency

A simple tests that may be done include a complete blood count, hemoglobin, checking the bilirubin level, and a reticulocyte count, which measures immature red blood cells, Heinz bodies may also be seen under microscope. Some other tests are also available to determine the deficiency or activity of enzyme G6PD (3). The other tests are given below:

Table 1. Comparison of test methods

Tests	Characteristics	Short incoming for field and mass-screening
Brilliant cresyl blue decolorizazion test	Involve the action of G6PD and NADPH diaphoresis. A deficiency of either one of these enzymes on RBCs would result in the brilliant cresyl blue remaining unchanged in the test.	Laborious process, require technical skills and has low sensitivity.
Methemoglobin reduction test	Based on the oxidation of hemoglobin to mathemoglobin by sodium nitrate and the subsequent enzymatic reconvertion to hemoglobin in the presence of methylene blue.	Laborious, qualitative, and low sensitivity. Does not enable identification of heterozygous deficient females.
Formazen ring test	Use the principle of the 3(4,5-dimethyl-2-thiazolyl)2,5-diphenyl-2H-tetrazolium bromide (MTT)-linked spot test when G6PD is present at normal levels, MTT is reduced to a purple insoluble formazan derivative and results in a specific diameter of discolourization.	Prone to misdiagnose ring thickness may be affected by exogenous factors.
Flouroscent spot test	International council of standardization in hematology (ICSH) recommended method.	Its cut off value for G6PD deficiency determination is only 10-20 percent of the normal G6PD activity, which exclude patient with moderate enzyme deficiency and increase the risk of false normal diagnose.

Causes

Red blood cell destruction can be triggered by infections, severe stress, certain foods (such as fava beans), and certain drugs (3).

Drugs That Can Trigger Hemolysis in G6PD-Deficient Children were:

1. Antimalarial (primaquine, maloprim, chloroquine)
2. Antihelminthes (beta-naphthol)
3. Antibacterial (chloromphenicol)
4. Analgesics (aspirin)
5. Sulphonamides

Method and material

Study design: Cross sectional study

Place of study

This study is conducted at civil hospital, National Institute of Child Health and Lady Dufferin Hospital, Karachi.

Subject and sample size

300 male childrens (age: day 1 to 7 years old) are screen for G6PD deficiency by doing the comparision between two methods (Formazan ring method and Dye decolorization method).

Inclusion criteria

This study is only performing on male childrens age from day1 to 7 years.

Exclusion Criteria

This study excludes females

Methods of blood collection in neonates and childrens

- Blood collection from the heel is performed for newborn screening. The medial and lateral parts of the underfoot are preferred.
- For childrens elder than one year venipuncture method is used similar to adults.

Formazan ring test

Principle of the Formazan ring method

The Formazan ring method uses the principle of the MTT Linked Spot Test recommended by the World Health Organization Scientific Group, with a minor modification. The reduction of NADP by G6PD is linked to the reduction of MTT, a soluble tetrozolium compound, to a purple (bluish) insoluble Formazan derivative with the use of a catalyst, Phenazine Methosulfate (PMS).

Method

Sample Preparation

Blood samples are drawn and mixed with an anticoagulant EDTA from newborn males and sent to laboratory where it is loaded on filter paper and hold up until air dried at room temperature.

Chemicals Used to Prepare Agar Plate

The chemicals which are used to make agar plate are:

- Glucose-6-phosphate disodium salt hydrate (G6PNa2)
- nicotinamide adenine dinucleotide oxidized form (NADP)
- phenazine methosulfate (PMS)
- 3(4,5-dimethyl-2-thiazolyl)2,5-diphenyl-2H-tetrazolium bromide (MTT)
- Tris HCL
- Magnesium chloride ($MgCl_2$)
- Agar

Gel thickness is approximately 3mm and is stored in refrigerator at 2-4 degree centigrade.

Procedure

A 3 mm disc was punched from the filter paper and placed on the gel surface, ensuring that the whole disc was completely in contact with the gel surface. The agar plate was wrapped in aluminum foil and incubated at 37°C for at least 8h. After the incubation period, the diameter of the Formazan blue ring was measured. A negative screen was defined as a bluish discoloration around the disc measuring more than 7 mm in diameter. A positive screen was defined as a disc with absence of bluish discoloration or a bluish discoloration measuring less than 7 mm.

Dye decolorization test

Principle of dye decolorozitaion test

The brilliant cresyl blue (BCB) dye test is best on the principle that NADPH formed through the action of G6PD reduces BCB to a colourless state. BCB serves two functions in the test: it serves as indicator as well as stimulant (4).

Method

Reagent

- Sodium g6pd
- NADP
- Tris buffer
- Distilled water
- Brilliant cresyle blue (BCB)

Procedure

- Take 0.02 ml of whole blood (anticoagulated with acid citrate dextrose ACD) and add in 1.0 ml distilled water to make dilution.
- The above given chemicals are mixed to make reagent.
- Now mix the diluted blood sample into the reagent.
- Top the mixture with oil to avoid the contact of test solution with air.
- Incubate at 37 degree centigrade in water bath.
- Observe the solution every 5-10 minutes after 40 minute for discolourization until the red colour of hemoglobin can be seen.
- Normal specimen will usually be decolorized at 65 minutes.
- Lack of decolourization shows the G6PD deficiency.

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3/7/2019