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Correlation between G6PD deficiency and some hematological parameters

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ABSTRACT

The most prevalent enzyme deficiency in the world, glucose-6-phosphate dehydrogenase deficiency (G6PD deficiency), is an inherited metabolic defect that increases the risk of hemolytic anemia. The purpose of this study to evaluate the methods used to detect G6PD Deficiency approved in our laboratories as the best marker to determine the deficiency of the G6PD enzyme. Fifty samples were collected from children suffering from hemolytic anemia had been diagnosed by G6PD deficiency. Complete blood count (CBC) had been done by automated hematology analyzer. G6PD enzyme activity is determined using spectrophotometry, which measures NADPH generated based on changes in the sample's absorbance at 340 nm over time. Reticulocyte count also was detected. From total of 50 child patients, 34 (68%) were male and 16 (32%) were Female with significant differences between them at **(***p value= 0.001*). The age group 0-2 years was the most prevalent group that has G6PD deficiency 21/50 (42%). The incidence of the disease in children between the ages of 2-4, 4-6, and under 6 years were 15/50 (30%), 8/50 (16%), and 6/50 (12%) respectively. From fifty participants in this study, 88% of them showed low hemoglobin levels, whereas, 100% of patients have low red blood cell count. Increasing of reticulocytes has been detected in 100 % of participants. 84% of patients revealed increasing in WBC count.

Keywords: hemolytic anemia, G6PD, Glucose 6-phosphate dehydrogenase, reticulocytes, Hb.

INTRODUCTION

The most frequent enzyme deficiency, glucose-6-phosphate dehydrogenase deficiency, causes a spectrum of disease, including neonatal hyperbilirubinemia, chronic hemolysis, and acute hemolysis. Anemia often develops when the blood's concentration of the protein hemoglobin decreases (Luzzatto,2006). It has many classifications based on several factors (Domingo, Satyagraha, Anvikar, Baird, Bancone, Bansil & Von Seidlein, 2013). In particular,

glucose-6-phosphate dehydrogenase deficiency, an X-linked hereditary condition that results in acute and chronic hemolysis, reduces glutathione levels, which leaves red blood cells more susceptible to oxidative damage and hemolysis (Berg, Tymoczko, & Stryer, 2007); (Warburg & Christian, 1932; Cappellini & Fiorelli 2008). It affects children more than adults (Pamba, Richardson, Carter, Duparc, Premji, Tiono, &Luzzatto, 2012).

The variant that causes chronic hemolysis is rare because it is related to sporadic gene mutation rather than the more common inherited gene mutation. Different gene mutations cause different levels of enzyme deficiency, with classes assigned to various degrees of deficiency and disease manifestation (Frank, 2005).

Newborns can be tested for G6PD deficiency, and delivery facilities should use these tests (Iranpour, Hashemipour, Talaei., Soroshnia &Amini, 2008). G6PD deficient erythrocytes do not generate NADPH in any other way than through the PPP (the pentose phosphate pathway) and for this reason they are more susceptible than any other cells to oxidative damage (Watchko, Kaplan, Stark., Stevenson, & Bhutani, 2013). Preventing hemolysis by limiting oxidative stress is the most effective way to manage G6PD deficiency (Cappellini & Fiorelli, 2008). As mentioned above, this disease is X linked to the chromosome and also often appears in childhood (Orkin, Nathan, Ginsburg, Look, Fisher& Lux, 2008).

Laboratory Diagnosis Depend on clinical story, clinical examination, laboratory tests. The tests that must be performed are: Complete Blood Count (CBC) test, Urine test and G-6PD enzyme Levels, Urine test; Significant intravascular hemolysis is indicated by decreased haptoglobin levels, hematuria, and the presence of urine hemosiderin (Ley, Bancone, Von Seidlein., Thriemer, Richards, Domingo& Price, 2017).

Spectrophotometry detects the generation of NADPH by comparing the sample's absorbance at 340 nm over time in order to calculate the G6PD activity (Ley,Winasti Satyagraha, Kibria, Armstrong, Bancone & Howes, 2022). Spectrophotometry is a reference method for a Qualitative test but it is not appropriate for frequent use, a cutting-edge handheld biosensor device has been created by SD Biosensor (G6PD STANDERD) (Thielemans, Gornsawun, Hanboonkunupakarn, Paw, Porn, Moo & Bancone, 2018). Simple and affordable, the fluorescent spot test is reliable for usage in many contexts and provides a result in under 30 minutes. The test is affirmative if the blood spot does not glow when exposed to UV light, however reagents must be kept in a cold chain, there must be electricity, and interpretation of the data requires training (Jiang, Ma, Song, Lin, Cao, Wu & Hsiao, 2003);(Mason, Bautista and Gilsanz, 2007).

In this study, light was shed on the emerging of the G6PD deficiency and its relationship to age and gender, the reasons that led to the high incidence of injuries, and the most important markers for diagnosis the disease.

Materials and Methods

On March 1, 2021, The Karbala Health Directorate's Ethical Committee approved the study protocol. Furthermore, the patients' verbal consent was obtained before taking the sample. During the sampling, precautions were taken to ensure the safety of the participants. This work was also carried out by the Iraqi Ministry of Health's Ethics Committee and followed all national rules.

On April 11, 2021, fifty samples were collected from children suffering from hemolytic anemia had been diagnosed by hematoglocytes G6PD deficiency. The collection of samples took place in Al-Jawadin Specialized Medical Laboratory in the Holy Karbala / Al-Iskan.

Three mL of blood was taken from a vein from each patient and placed in a tube containing the anticoagulant EDTA for use in CBC, Reticulocyte count, and G6PD enzyme tests.

Complete blood count (CBC) had been done by automated hematology analyzer. G6PD enzyme level detected by Spectrophotometry that detect the level of G6PD activity by measuring the formation of NADPH based on the difference in absorbance of the sample at 340 nm over time.

Reticulocyte count in a tube, equal quantities mixed (one drop of blood sample + one drop of dye (methylene blue). Then, the mixed tube putted in the incubator for 15 minutes at a temperature of 37°C, and made a film on a glass slide. After drying the film, examine it with a microscope using an oil lens. Finally, count the retinal cells and red blood cells in ten fields, and following equation applied: Reticulocytes = total number of reticulocytes / total number of red blood cells x 100.

Results and Discussion

From total of 50 child patients, 34 (68%) were male and 16 (32%) were Female with significant differences between them at (p value= 0.001) [Figure 1].

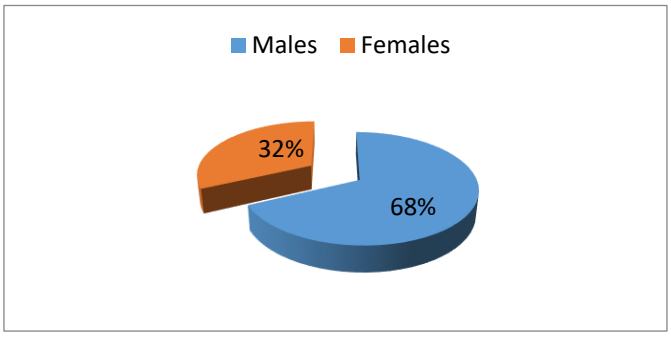

Figure 1: Distribution of G6PD deficiency among genders

The age of patients ranged from 17 to 90 months (1.4 to 7.5 years) with mean 38.06± 22.79. The age group 0-2 years was the most prevalent group that has G6PD deficiency 21/50 (42%). The age groups of 2-4 years, 4-6 years, and > 6 years were 15/50 (30%), 8/50 (16%), and 6/50 (12%) respectively. [Figure 2]

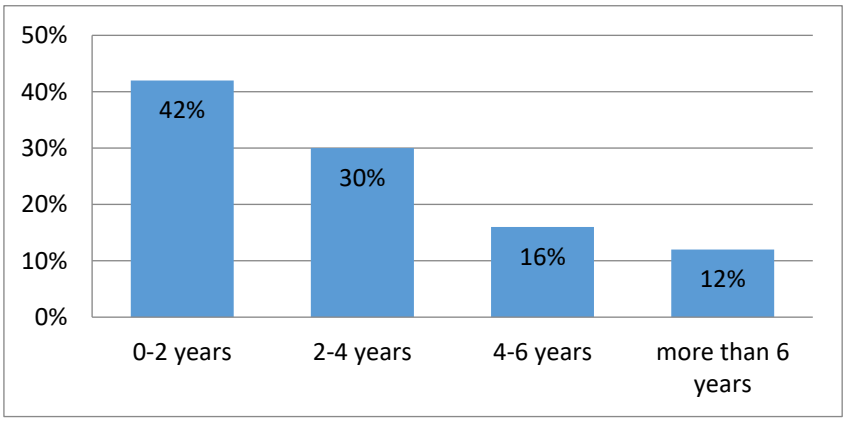

Figure 2: Distribution of G6PD deficiency among age groups

The mean of distribution of G6PD level among gender showed that distribution among male was 54.76± 25.29, while in females were 50.50± 25. 22. So no significant differences have been observed at (p value= 0.58)

From fifty participants in this study, 88% of them showed low hemoglobin levels, whereas, 100% of patients have low red blood cell count. Increasing of reticulocytes has been detected in 100 % of patients with G6PD deficiency. 84% of patients revealed increasing in WBC count.

The most prevalent flaw in red blood cells is glucose 6-phosphate dehydrogenase (G6PD), which is encoded by an X-linked human gene (Xq2.8) Red blood cells (RBCs) with a G6PD deficiency have been extensively studied. The pentose phosphate pathway is the only known

source of NADPH, which allows RBCs to balance the oxidative stress brought on by various oxidants while maintaining the reduced form of glutathione (GSH). The ratio of reduced to oxidized GSH in healthy RBCs is 100:1. If NADPH levels cannot be kept high, as in the case of G6PD deficiency, the GSH levels decrease and oxidative damage takes place, culminating in acute hemolysis (Cappellini & Fiorelli, 2008).

from scientific side, testing for G6PD deficiency should be recommended whenever an acute hemolytic reaction is carried on by exposure to a known oxidative drug, an infection, or the consumption of fava beans, whether in children or adults, especially if they are from the African, Mediterranean, or Asian regions. Additionally, members (especially males) (Zekavat, Makarem, Bahrami., Dastgheib &Dehghani, 2019).

50 samples from the current study were taken, with 68% of them being male and 32% being female. Males are more sensitive and have a higher incidence of the disease since it is associated with the X chromosome. Thematic analysis of qualitative data has been done using the content analysis method. One study recommended using quantitative G6PD-level assessment rather than customary qualitative techniques to diagnose common G6PD insufficiency (Mast, Blinder, Flax, Dietzen, 2002).

In the current study, 88% of patients showed low hemoglobin levels. Since RBCs have a lifespan that reflects bone marrow iron concentration from up to 120 days ago, Hgb measurements frequently miss situations of early or mild iron shortage. 12% of case were normal because high Retic count (Reticulocyte have 6 time more enzyme than normal RBCs). Reticulocyte hemoglobin content (RHC) provides a more accurate "real-time" test of bone marrow iron status since reticulocytes only survive in the peripheral for one or two days (white 2005). On the other hand, iron deficiency is not always the root cause of childhood anemia. As a result, the measurement of a single Hgb level could lead to needless treatment and testing (Ullrich, Wu, Armsby, Rieber, Wingerter, Brugnara & Bernstein, 2005).

In this study the measurement of RBC was low in 100% of the participants and this is a good indicator that can be inferred to the presence of a defect. RHC measurement could help prevent this problem. An RHC of less than 27.5 pg was 83 percent sensitive in detecting iron deficiency in a study of infants aged nine to twelve months, whereas a Hgb level of less than 11 g per dL (110 g per L) was only 26 percent sensitive (Beutler, Blume, Kaplan, Löhr, Ramot & Valentine, 1977). More research is required to evaluate whether screening with this test is clinically and financially advantageous because RHC is not available in all laboratories.

There was an increase in the number of WBC, where the number of patients who increased it was in 84% of patients, while the normal among children is 5000-10000 g/L (Most of the previous studies do not use elevated white blood cell counts as a strong predictor for detecting

Except for some studies that use the spectrophotometric assay which is the best method for detecting G6PD in laboratories, As for the Reticulocyte test, it is either used for anemic patients or for iron deficiency anemia, in this study, Retic count was increase in all patients with (G6PD) deficiency but The reticulocyte analysis among patients showed the increased number with low degree of maturation in both IDA (iron deficiency anemia) and G6PD def. patients (Butthep, Wisedpanichkij, Jindadamrongwech, Kaewkethong, Pattamakom, Sila-Asna, & Bunyaratvej, 2000).

In this study, most of the age groups that came to the diagnosis are from the ages ranging from 1.4 to 7.5 years like most studies, the reason may be due to the exposure of this age group to the factors that lead to the appearance of the symptoms of the disease for the first time due to the lack of knowledge of the parents about the existence a hereditary genetic defect arising from mutations in the *G6PD* gene.

Most of the patients who underwent these tests had recently been exposed to factors that affect the appearance of symptoms like Fava beans, some drugs and infections. While, the other patients were chronically and they usually do a regular checkup.

CONCLUSIONS

Examinations must be done for those most at risk since hemolytic anemia can be linked to acute renal failure, which can happen at any age and is potentially fatal. When an acute hemolytic reaction is brought on by exposure to a known oxidative medication, an infection, or ingestion of fava beans in either children or adults, testing for G6PD deficiency should be recommended. Male children are more likely than female children to have a G6PD deficit (p value = 0.001). The most common age range for G6PD deficit was 0–2 years, while children older than 6 years showed the lowest age range for G6PD deficiency. Low red blood cell count has been detected in all patients. On the other hand, high reticulocytes count has been detected in all patients. Those results may be good markers that can be inferred to the presence of a defect. Since acute renal failure can develop at any age and is associated with chronic and frequent hemolytic anemia, it is important to conduct tests on those who are most at risk. When an acute hemolytic reaction is follows exposure to a known oxidative medication, an infection, or the consumption of fava beans, whether in children or adults, testing for G6PD deficiency should be recommended. Most of the age groups that came to the diagnosis are from the ages ranging from (11-20), (21-30) month as in most studies. The age group 0-2 years was the most prevalent group that has G6PD deficiency, while child more than 6 years showed the less age group suffering from G6PD deficiency. Etic count was increase in all patients with (G6PD) deficiency .The WBC count could not be adopted in the diagnosis as

there were 17 of the participants had normal white WBC counts which is equivalent to 34%.Because RBCs have a lifespan that reflects bone marrow iron levels from up to 120 days ago, Hgb measurements frequently miss cases of early or mild iron shortage.RBC was low in 98% of the participants and this is a good indicator that can be inferred to the presence of a

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