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Role of Parents' Information and Screening Test Sensitivity in Acute Attack of G6PD Deficiency

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Abstract

Background: The current study aimed at surveying the screening test accuracy for G6PD (glucose-6-phosphate dehydrogenase) deficiency and the patients' parents information about the disease progression and persistence as determinant factors in hospital admissions of these patients in Shiraz, Southern part of Iran. Methodology: This study was conducted on 239 subjects with the final diagnosis of G6PD deficiency disease from April 2013 to July 2014. The data were obtained through their old chart review and also call interview with their parents. Patients' first hemoglobin level documented in the hospital was considered as the severity marker of the disease. Moreover, the numbers of packed cell RBC transfusions as the probable marker of illness complications and severity were recorded. Results: In the current research, 59.8% of parents were aware of their children`s disease diagnosis before hospital admission. The sensitivity of disease screening test (Fluorescent Spot Test) has been about 59.8% in the selected population. Most of the patients (41.84%) have been admitted with hemoglobin concentration between 5&7 mg/dl, whereas 50.2%, 23.43%, and 7.1% of them have had one, two and more than two times blood transfusion, respectively. Conclusions: The results of this study showed that parents' information about the disease transient etiology and its improvement with aging were the main reasons for neglecting the condition. Due to complications of disease acute attacks, improvement of illness screening test sensitivity for earlier detection of the patients and screening centers of staff recommendations to take caregivers teachings about the persistent disease etiology and triggering factors should be considered more seriously.

Key words: Glucose-6-phosphate dehydrogenase Deficiency, Parents, Sensitivity, Screening, Acute Attack.

Introduction

The most common enzymopathy of red blood cells (RBCs) in humans, Glucose 6-phosphate dehydrogenase (G6PD) deficiency, is an x-linked disease affecting 200 to 400 million peoples worldwide (Mason, Bautista and Gilsanz, 2007; Glader et al., 2004).

Prevalence

It is more common in African-Americans and also in people whose families originally came from the Mediterranean area and the Middle East. G6PD deficiency is present in approximately 20 percent of South African black males. (Bienzle, 1981)

According to WHO, the prevalence of G6PD deficiency in Iran is between 10-14%; however, other studies have reported the prevalence of 1 to 22.8%. (Moosazadeh, Amiresmaili and Aliramezany, 2014; Noori-Daloi et al., 2007)

Meta- analysis conducted by Nkhoma et al. (2009), reported a total prevalence of G6PD deficiency of 4.9% in the world.

G6PD deficiency is expressed in males carrying a variant gene, while heterozygous females are usually asymptomatic (Van Noorden et al., 1982, 1989). However, the RBC enzyme activity in heterozygous females differs depending upon the degree of ionization and the abnormal G6PD variant expression (Hsia et al., 1993).

Pathogenesis and variants of the disease

The G6PD enzyme helps red blood cells to confront different oxidative stress. The amount of G6PD enzyme determines a likelihood of developing hemolysis and severity of the disease (Glader et al., 2004).

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The disease expression encompasses a broad spectrum of hemolytic syndromes such as acute hemolysis, fauvism, congenital non-spherocytic hemolytic anemia, & neonatal hyperbilirubinemia (Nantakomol et al., 2013). According to the magnitude of the enzyme deficiency and the severity of hemolysis, G6PD variants are categorized into five classes. Class I variants have severe enzyme deficiency and chronic hemolytic anemia.

Class II variants (such as Mediterranean G6PD) also have a severe enzyme deficiency but have only intermittent hemolysis. While Class III variants (such as G6PD A-) have moderate enzyme deficiency with intermittent hemolysis usually associated with infections or medicines. Class IV variants, G6PD B, have no enzyme deficiency or hemolysis. This class is seen in most Caucasians, Asians, and a majority of blacks. Finally, Class V variants have increased enzyme activity.

The Mediterranean (a class II variant with enzyme half-life measured in hours) & A- subtype (a class III variant with a half-life of 13 days [normal 62 days]) are the most common types of G6PD deficiency. They are usually asymptomatic, (Brewer, 1961) but a sudden destruction of enzyme deficient erythrocytes can be triggered by certain drugs, fava beans ingestion, some infections and rarely by metabolic abnormalities (e.g., diabetic ketoacidosis).

Infection is probably the most common factor triggering hemolysis in G6PD-deficient cases. (Burka, Weaver and Marks, 1966; Shannon and Buchanan, 1982) In one study, it has been affirmed that hemoglobin concentration decreased abruptly in approximately 20 percent of G6PDdeficient subjects with pneumonia (Burka ER, Weaver Z, Marks, 1966).

Fauvism

G6PD Mediterranean is the G6PD variant most commonly implicated in Fauvism. Thus, fauvism occurs most often in Italy, Greece, North Africa, and the Mid-East people (Beutler, 1994). Fauvism occurs most commonly in children, primarily males, between the ages of 1 to 5. It can be caused in breastfed children if the lactating mothers eat dry, fresh beans or bean products, including tofu and soy sauce.

Manifestation

The disease presents its symptoms within 5 to 24 hours after fava bean ingestion with acute intravascular hemolysis manifestations.

Mediterranean G6PD is the major variant accompanied by acute hemolysis, which is usually mild. However, massive intravascular hemolysis resulting in nephrotoxic acute renal failure occurs occasionally (Whelton, Donadio and Elisberg, 1968; Phillips and Silvers, 1969). A headache, nausea, back pain, chills, and fever are followed by hemoglobinuria and hyperbilirubinemia.

Complications, Treatment, and screening

Treatment of the patients with glucose 6-phosphate dehydrogenase (G6PD) deficiency is determined by the clinical syndrome with which it is correlated. The hemoglobin concentration falling is acute, and often, in the absence of transfusion, can be fatal. Therefore, red cell transfusion may be necessary that poses significant risks such as infectious agent transmission and various immunologic reactions. The rate of transfusion reaction in children is approximately 1 percent, as illustrated in a multicenter study from the Pediatric Health Information System of 44,632 hospitalized children (18 years of age or younger) who received RBC transfusions (Slonim et al., 2008). The patients with hematologic and oncologic diseases such as G6PD-deficient patients are more susceptible to show these adverse effects (Brittingham and Chaplin, 1957; Heddle et al., 1993). A Canadian study estimated that the incidence of severe septic episodes with transfusion of blood components is 1 in 500,000 red blood cell units (Blajchman and Goldman, 2001). The estimated incidence of transfusiontransmitted bacterial infection (TTBI) is higher than the expected rate for transfusion-transmitted HIV or hepatitis C transmission (approximately 1 in 2 million units transfused) (Vasconcelos and Seghatchian, 2004; Goodnough, Shander and Brecher, 2003; Jacobs, Palavecino and Yomtovian, 2001).

The most important point in G6PD deficient patients is a diagnosis of the disease via reliable screening tests as soon as possible and prevention of oxidative stress exposure in the patients. Diagnosis of illness depends on some screening tests such as FST (Fluorescent spot test) and quantitative fluorescence assay. Fluorescent spot test is a qualitative method, and the accuracy of the results is operator dependent. The advantage of this approach is that it does not require expensive equipment. Although the FST can identify severe deficiencies, discrimination of intermediate levels with this test is more difficult. Quantitative fluorescence assay has high sensitivity, and the results are objective and accurate. It discriminates mid to normal levels of the G6PD enzyme with fine resolutions. Now, fluorescent spot test is the most common test performed in the neonatal period and whenever that the disease is suspicious. The detection rate for both methods is 100% for the samples from male patients and all the female homozygous patients (Jiang et al., 2014). The only way to identify heterozygous females precisely for G6PD is through genotyping or intracellular G6PD activity cytochemical staining.

Cytochemical staining includes microscopic visualization or enumeration (by flow cytometer) of the two distinct red cell populations resulting from the G6PDnormal and deficient allele expressions (Larue et al., 2014; Ainoon et al., 2003; Johnson et al., 2009).

Materials and Methodologies

The present study, as a retrospective, cross-sectional study, evaluated 239 subjects admitted to Shahid Dastgheib hospital in Shiraz, from April 2013 to July 2014 with final diagnosis of G6PD deficiency by positiveG6PD deficiency screening test via FST test with Kimiapajohan company kit (92606). The data were obtained via their old chart review and also call interview with their parents. As a severity marker of the disease, the patients' first documented hemoglobin levels in the hospital were obtained from their old charts. In addition, their caregivers' information about the patient's history of the disease has been achieved via their old charts review (with the review of past medical history of the patients in their old chart). The parents' reason for permitting the patients to be exposed to triggering factors was questioned through call interview (with asking this question that why the parents have ignored their previous information about their children disease). The numbers of packed cell RBC transfusion of the G6PD deficient patients were recorded because of their importance in showing the degree of illness severity and also as a complication of the disease. These data were gathered from the patients' old chart review.

Result:

The current study had evaluated 239 cases. Out of which, 107 cases (about 44.7%) were female and 132 cases (55.3%) were male. In other words, female to male ratio was about 0.8 that was significant considering the disease x-linked etiology. Age distribution of the sample was as below: in both extremes of age, 7.1% of them (17 cases) were below 2 years old and about 30.54% (73 cases) were 7 years old or higher. Most of the cases (149 cases or 62.3% of them) were between the age of two and seven years old.

One of the most important complications of G6PD deficiency is acute hemolytic anemia that can be life threatening so necessitates blood transfusion. Therefore, the first documented hemoglobin levels in the hospital were reviewed. Among which, 22 cases (9.2%) have had hemoglobin level below 5mg/dl. Most of them, namely 100 cases (41.84%), have been admitted with hemoglobin concentration between 5 and 7mg/dl. Additionally, 35.56% (85 cases) have had hemoglobin level between 7 and 9mg/dl. Only 32 cases (13.3%) had hemoglobin concentration equal or higher than 9mg/dl.

Unfortunately, majority of the patients' parents were informed about their children disease diagnosis before the hospital admission. To mention, 143 of 239 parents (59.8%) have had neglected their children`s disease by permitting them to be exposed directly or indirectly to Fava beans. In simpler terms, G6PD deficiency screening test sensitivity was about 59.8% in our sample that was significantly low in comparison to other studies` results. The test sensitivity was calculated by considering our population (239 cases) as certain patients that has been documented through conducting FST for them with Kimiapajohan company kit (92606), and 143 cases of them have been known cases of the disease with a positive screening test. Consequently, the screening test was successful in detection of 59.8% of the patients. One of the most significant findings of the study was the number of the patients who had received blood. 193 of 239 cases (about 80.7%) have had received at least one-time transfusion. Whereas 120 cases (50.2%) have had a one-time blood transfusion, 56 cases (23.43%) had 2 times transfusion. It is significant that 7.1% of them (17 cases) had more than 2 times transfusion. These facts and figures were so important considering the blood transfusion adverse effects such as infections transmission risk.

Table 1: Hemoglobin level distribution

Hemoglobin level	< 5 mg/dl	5-7 mg/dl	7-9 mg/dl	> 9 mg/dl
Number of the patients	22	100	85	32
Percentage of the patients	9.2 %	41.84 %	35.56 %	13.3 %

Table 2: Transfusion condition

Transfusion condition	Without transfusion	One time transfusion	2 time transfusion	> 2 times transfusion
Number of the patients	46	120	56	17
Percentage of the patients	19.3 %	50.2 %	23.43 %	7.1 %

Discussion and Conclusion

According to the current study`s results, G6PD deficiency screening test sensitivity was about 59.8%. In fact, 40.2% of the patients have not been recognized via neonatal screening test. As mentioned in the review section of articles, the sensitivity of G6PD deficiency screening test was significantly low in our study in comparison to other studies. In the paper of J. Jiang1 et al. (2014), no false-negative results about G6PD deficiency screening tests were found although they had used a qualitative screening test. Moreover, in a study

conducted by Nicole Larue et al. (2014), the sensitivity of the qualitative test was at least 82.6% &91.3 % (respectively for the BinaxNOW assay & FST test) in cut-off point of 60% (that shows RBC enzyme deficiency in comparison to normal RBC).

One of the most common reasons can be attributed to the fact that these patients may first present neonatal hyperbilirubinemia. Shortly after a hemolytic event, and at a time when a reticulocyte response has occurred, G6PD activity may be normal, secondary to the fact that G6PD activity is higher in remained reticulocytes. So a false-negative screening test may be found. A repeated examination several weeks later, when the reticulocytosis has abated, may be necessary to confirm the diagnosis. The difference in the disease attack with the same G6PD variant is believed to be related to local customs and variations in oxidant exposure. All affected individuals should avoid exposure to medicines known to trigger hemolysis whenever possible. It has also been suggested that the affected individuals should refuse the ingestion of fava beans, which can cause hemolysis in some of them.

Considering this fact that majority of the patients' parents have had neglected the diagnosis of disease (59.8% of the patients were known cases of the disease), the reason was asked in call interviews. The most common reason was their misunderstanding about the disease. In fact, they thought G6PD deficiency is a transient illness, despite the fact that some of the hospital staff have expressed that the condition would improve with aging. Therefore, the parents have permitted their children to be exposed to fava beans. Still another study was found considering on G6PD deficient patients' caregivers' information about the disease as well as its important role in the illness attack prevention.

As mentioned above, the hemoglobin concentration fall in G6PD deficient patients can be so acute and significant that needs blood transfusion. Currently, each unit of donated blood in the United States is screened for some infections, including HIV, hepatitis B, and C viruses (HBV & HCV), human T-cell leukemia virus, West Nile virus, Trypanosomacruzi, and Treponema pallidum. While the risk of acquiring infection is low due to donated blood screening for TTBI, some risks remain that are largely from "window period" donations, during the time when antigen or antibody are at such a low level that current techniques cannot detect them. In our country, in Iran, screening tests were conducted only for HIV, HBV & HCV infections. It should be considered that HIV is transmitted in about 93% of blood transfusions involving infected blood. (Baggaley et al., 2006) (Also see Table 3) While in developed countries the risk of acquiring HIV from a blood transfusion is tiny (less than one in half a million), in low-income countries, only half of the transfusions may be appropriately screened (as of 2008) (W.H.O., 2011). It is estimated that up to 15% of HIV infections in these areas come from transfusion of infected blood and blood products, representing between 5% and 10% of global infections. (Rom and Markowitz, 2007)

This information has made us pay more attention to G6PD deficiency. For the first step, its screening test improvement or repeated tests shortly after the first negative neonatal screening test is advisable (Reclos et al., 2007, Shah et al., 2012). The patients' family training about the disease, especially its trigger factors, is so beneficial and cost-benefit wise. This task is a duty of the health center staff when confronting a new case of G6PD deficiency. With effective training, most of the unwanted admissions and therefore their consequences can be inhibited. At the global symposium on neonatal jaundice and nuclear jaundice in 2005, it was proposed that at discharge, parents should be made aware of the implications of the disease, and long-term care and follow-up treatment should be provided to the patients by the hospital. Seemingly, this misunderstanding should be corrected via training the parents of G6PD deficient patients as soon as possible after the diagnosis of disease, especially after the positive neonatal screening test. In other words, screening center staff should be advised to give caregivers instructions about the persistent disease etiology and triggering factors more seriously.

Table 3: HIV Transmission route

Average per act risk of getting HIV by exposure route to an infected source	
Exposure route	**Chance of infection**
Blood transfusion	90% (Smith et al., 2005)
Childbirth (to child)	25% (Coovadia, 2004)
Needle-sharing injection drug use	0.67% (Smith et al., 2005)
Percutaneous needle stick	0.30% (Kripke, 2007)
Receptive anal intercourse*	0.04–3.0% (Dosekun and Fox, 2010)
Intertie anal intercourse*	0.03% (Cunha, Burke, 2012)
Receptive penile-vaginal intercourse*	0.05–0.30% (Dosekun and Fox, 2010; Boily et al., 2009)
Intertie penile-vaginal intercourse*	0.01–0.38% (Dosekun and Fox, 2010; Boily et al., 2009)
Receptive oral intercourse*§	0–0.04% Dosekun and Fox, 2010)
Intertie oral intercourse*§	0–0.005% (Rom and Markowitz, 2007)
* assuming no condom use § source refers to oral intercourse performed on a man	

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