Screening For Glucose 6-Phosphatase Dehydrogenase Among Neonates At Wad Medani Obstetrics And Gynecology Teaching Hospital, Gezira State-Sudan (2015–2016)

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ABSTRACT
Background: Glucose 6-phosphatase dehydrogenase (G6PD) deficiency is the most common enzymatic disorder of red blood cell in humans. It is estimated that about 400 million people are affected by this deficiency (Glader, 2008). In 1956, Carson and colleagues discovered that individuals developing hemolytic anemia caused by the anti-malarial drug primaquine had a very low level of (G6PD) activity in their red blood cells (Carson, et al. 1956,Beutler, 1959). G6PD deficient may be also associated with neonatal jaundice, that when severe and untreated may lead to kernicterus. Objective: This is prospective descriptive cross sectional hospital based study was carried out with the aim of evaluating the incidence of G6PD deficiency among neonate in Wad Medani Obstetrics and Gynecology teaching Hospital. Materials and Methods: Cord blood samples from 85 new born during (2014-2015) were examined for G6PD activity by methaemoglobin reduction test (dye reduction test), the deficient samples were confirmed using quantitative assay. Result: The study revealed that 5.9% of examined participants were G6PD deficient which represent 4.7% of four males and 1.2% of one female out of 85 (100%) neonates with strong significant between the deficient group and rural area (P value: 0.000) particularly in those who was family history in 20 % of the deficient group. Conclusion: The study revealed that the prevalence of Glucose 6-phosphatase dehydrogenase was remarkable in 5.9% of the participants. So the study recommended to do neonatal screening for G6PD deficiency because it is a useful test for preventing and early treatment of complications associated with G6PD deficiency.

KEYWORDS: G6PD, X linked recessive, hemolytic crisis, kernicterus, neonate, Cord blood, methaemoglobin reduction test, quantitative assay.

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I. INTRODUCTION AND LITERATURE REVIEW

Glucose 6-phosphatase dehydrogenase (G6PD) deficiency is the most common enzymatic disorder of red blood cell in humans. It is estimated that about 400 million people are affected by this deficiency (Glader, 2008). In 1956, Carson and colleagues discovered that individuals developing haemolytic anaemia caused by the anti-malarial drug primaquine had a very low level of (G6PD) activity in their red blood cells (Carson, et al. 1956,Beutler, 1959). G6PD catalysis the first reaction in the pentose phosphate pathway, in which glucose is converted into the pentose sugars required for glycolysis and for various biosynthetic reaction. The pentose phosphate pathway also provides reducing power in the form of NADPH (Tsai, et al. 1998). The reduced form of glutathione is essential for the reduction of hydrogen peroxide and oxygen radicals and the maintenance of hemoglobin and other red blood cell proteins in the reduced state. (Luzzatto, 1995). G6PD is also necessary to the reduced form of glutathione that is produced with one molecule of NADPH (Tsai, et al. 1998). The reduced form of glutathione is essential for the reduction of hydrogen peroxide and oxygen radicals and the maintenance of hemoglobin and other red blood cell proteins in the reduced state. (Luzzatto, et al. 2001). The gene that codes for G6PD is located in the distal long arm of the X chromosome at the Xq 28 locus. The G6PD gene is 18 kilo bases (kb) long with 13 exons, and the G6PD enzyme has 515 amino acids (Yang, et al. 2011).

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G6PD deficiency is an X-linked recessive disorder with an inheritance pattern similar to that of hemophilia and color blindness; males usually manifest the abnormality and females are carriers. (Frank, 2005). Deficient G6PD alleles are distributed worldwide, a conservative estimate is that at least 400 million people carry a mutation in the G6PD gene causing deficiency. The highest prevalence of G6PD is reported in Africa, southern Europe, the Middle East, South East, and the central and southern pacific Island. (Frank, 2005). Most G6PD deficient individuals are asymptomatic throughout their life, and unaware of their status. The illness generally manifests as acute haemolysis, which usually arises when red blood cells undergo oxidative stress triggered by agents such as drugs, infection, or the ingestion of fava beans (Cocco, et al. 1998). G6PD deficiency usually present as drug-induced or infection induced acute haemolyticanaemia, favism, neonatal jaundice, or chronic non-spheroctytichaeomolyticanaemia. Several clinical disorder, such as diabetes and myocardial infarction (Shalev, et al. 1984), and strenuous physical exercise, have been reported to precipitate haemolysis in G6PD-deficient individuals (Ninfali, et al. 1995). G6PD deficiency is the most common human enzyme defect, being present in more than 400 Million people worldwide (Cappellini, et al. 2008) in 2010 it resulted in about 4000 deaths globally. (Lazano, 2012).

G6PD deficiency in widely disseminated throughout Africa, the Mediterranean basin, the Middle East, South East Asia, and Indigenous population of the Indian subcontinent. G6PDA is common in Africa. The Mediterranean variant in southern Italy, Sardina and other places around the Mediterranean base in and G6PD canton in southern China. This distribution of the deficiency equates with areas where plasmodium falciparum malaria is common, and this is thought to be evolutionary drive that produced such wide spread polymorphisms. It has subsequently been confirmed that G6PD deficiency does indeed protect against lethal falciparum malaria, particularly in childhood, and this protection, especially in hyper endemic areas, more than outweighs the haematological problems associated with deficiency. (Hoffbrand, et al. 2005)

According to the study done by world health organization (1989) the prevalence of G6PD deficiency in worldwide; 300 – 400 million people carry at least one deficient G6PD gene. (WHO, 1989). In other study done by NKhoma et al, 2009) G6PDA is most prevalence mutation in African and Afro-Americans. This class III mutation has a gene frequency of 11%. G6PD B (Mediterranean) is a more severe class II deficiency. This mutation is commonly found in population living in or originating from round the Mediterranean sea. The prevalence of G6PD Mediterranean varies greatly; frequencies of 2-20% are found of G6PD deficiency (70%) is found in Kurdish Jews (NKhoma, et al. 2009). Study done by RA-Basouni and T.SM.Taha in Sudan, about 7-5% of world's population caring a gene for G6PD deficiency, the proportion ranging from maximum of 35% in parts of Africa to 0.1% in Japan and Part of Europe.

II. OBJECTIVES

General Objective
To Screening of glucose-6-phosphate dehydrogenase (G6PD) among neonates in Wad Medani obstetrics and Gynecology hospital.

Specific Objectives
- To screen neonates for G6PD deficiency using methaemoglobin reduction test (qualitative test).
- To confirm the diagnosis in deficient subjects using commercial kit "Quantitative test"
- To find out the residence of the patients, family history and gander of the new born.
- To compare with residence of the pts, family history, and gander of the newborns

III. MATERIALS AND METHODS

Study Area
This study was conducted in Wad Medani Obstetric and Gynecology Hospital, the largest specialized hospital in the city and faculty of Medical laboratory Sciences, Gezira university.

Study Design
This is descriptive, cross-sectional, hospital based study on screening of G6pD deficiency in cord blood sample from neonate in Wad Medani Maternity Obstetric and Gynecology Hospital in Gezira State.

Study Population
The population of this study were neonates which were product of either normal vaginal delivery, elective or emergency cesarean section in Wad Medani Maternity Teaching Hospital.

Sample Size
The sample size was calculated according to formula:

\[ n = \frac{t^2pq}{d^2} = 103 \text{ samples} \]

\[ n = \text{sample number} \]

\[ t = \text{Standard value (95%) = Confidence level = 1.96} \]

\[ p = \text{The incidence = 7% as reported in post graduate hematology. Fifth edition.} \]
Inclusion and exclusion criteria

Exclusion Criteria
- Neonate with bilirubin that appear on the first day of their delivery.
- Neonate with evidences of intravascular and extravascular haemolysis.

Sample collection
After delivery of the baby, by either normal vaginal or sectional delivery, the cord sample was clamped temporarily into sterile syringe before delivery of the placenta and the sample transfer immediately into anticoagulant containers (EDTA).

Data Collection
Data were collected by a carefully designed questionnaire and direct mother interview. The questionnaire was designed for the purpose of this study.

Ethical Clearance
The ethical clearance was obtained from the Ministry of Health authority and permission from hospital. Consent was obtained from all new born's mothers.

Methods
Sample were treated and investigated in faculty of Medical laboratory sciences in haematology department. There are two stages in the diagnosis of red cell enzyme defect.

First: Screening procedures by methaemoglobin reduction test depend on Daci and Lewis practical haematology.

Second: Specific enzyme assays e.g. quantitative assays of enzyme by commercial kit from Saudi Arabia (United Diagnostics Industry Dammam 31413. K.S.A).

Methaemoglobin reduction test:

Principle
Sodium nitrate converts Hb to Hi, when no methylene blue is added, methaemoglobin persists, but incubation of the samples with methylene blue allows stimulation of the pentose phosphate pathway in subjects with normal G6pD level the Hi is reduced during the incubation period. In the G6pD-deficient subjects the block in the pentose phosphate pathway prevents this reduction.

Procedure
- Firstly the working reagent were prepared (sodium nitrite, methylene blue).
- Anticoagulated blood (EDTA) was used and sample tested preferably.
- 500μL of blood was added to the tube containing 50μL of the combined reagent (sodium nitirte, methylene blue), then the tube was closed by stopper and mixed the contents were gently by inverting it 15 times.
- Control tubes were prepared by adding 500μL of blood to a similar tube without reagent (Normal Reference) and to tube containing 50μL of sodium nitrite dextrose mixture without methylene blue (deficient reference tube).
- The sample was incubated at 37°C for 90 min "waterpath"
- After the incubation 50μL volumes was pipetted from the test sample, the normal reference tube and the deficient reference tube into 5ml of distal water in separated. Then the colours were compared in the different tubes after content was mixed.

Interpretation
Normal blood yields a colour similar to that in the normal reference tube "clear red". Blood from deficient subjects gives a brown colour similar to that in the deficient reference tube. Heterzygote give intermediate reaction.

Quantitative assay of the enzyme:

Principle
The enzyme G6PD (old name G6P-DH) catalyses the dehydrogenation of glucose 6-phosphate as the first step in pentose phosphate pathway. NADP+, the electron acceptor, is reduced to NADPH in the reaction. The rate of increase of optical density that occurs with the formation of NADPH from NADP in blood hemolysate is measured at 340 nmm spectrophotometer. (Warburg, et al, 1932).

Procedure
- The number of the erythrocytes was Count per mL of blood.

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200μL of blood was washed three times with 2ml aliquots of saline (0.9% NaCl) by centrifugation for 10 minutes at approximately 3000rpm, and then the saline layer was removed completely without any loss of erythrocytes.

The erythrocytes were resuspended in 500μL of lysing reagent (G6p-DH lysing reagent) and incubated for 10 minutes at room temperature and then immediately centrifuged for 3 minutes at 300rpm, 50μL of supernatant hemolysate was used as a sample for erythrocytes activity determination.

In another test tube, 3ml of buffer (G6PDH buffer) was pipetted and 100μL of NADP reagent was added.

To that mixture, 50μL of haemolysate prepared in step 1 was added and incubated for further 5 minutes at room temperature.

To the above mixture 50μL of substrate (G6PDH substrate) was added and after 30 seconds initial absorbance was read at 340nm against distilled water by spectrophotometer, and repeated absorbance reading every minutes for the next 3 minutes and the mean A/min was calculated.

**IV. CALCULATION:**

\[
\Delta A/\text{min} \times 30868 = \text{G6PDH activity in } \mu\text{m/erythrocytes ml of blood.}
\]

\[
\therefore \text{G6PDH activity} = \frac{\Delta A \times 30868}{\text{RBCs Count}} \text{ "factor"}
\]

**Interpretation**

The results were interpreted according to normal reference value for normal control which were 80 - 180μm/10⁹ cells.

**Unit Definition**

In unit of the enzyme activity is the amount of enzyme that will convert 1mmol of glucose 6-phosphate per minute to 6phosphogluconate under the specified condition of reaction.

**V. RESULTS AND DISCUSSION**

This study was carried at Wad Medani Obstetrics and Gynecology Teaching Hospital and Faculty of Medical Laboratory Science, conducted in the period from (2015-2016). A total of 85 subjects were screened for glucose 6-phosphate dehydrogenase deficiency.

**Characterization:**

- **Residence:**

  According to Residence in this study, 67 (78.8%) of study sample from rural area, while only 18 (21.2%) of them from urban area. (Table & figure 1).

<table>
<thead>
<tr>
<th>Residence</th>
<th>Rural</th>
<th></th>
<th>Urban</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>67</td>
<td>78.8%</td>
<td>18</td>
<td>21.2%</td>
<td>85</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table & figure (1):** Distribution of the study sample according to their residence.

**Figure 1:**

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- **Consanguinity of Parents:**
  Regarding to the consanguinity of parents (24.7%) of the subjects showed 1st degree relative, (29.4%) 2nd degree relative while less than half (45.9%) of the study sample showed no relation between the parents. (Table 2).

| Table (2): Distribution of the study sample according to their consanguinity of parents |
|---------------------------------|---------|---------|---------|---------|
| consanguinity of parents | 1st degree relative | 2nd degree relative | No relation | Total |
| No % | No % | No % | No % | No % |
| 21 | 24.7 | 25 | 29.4 | 39 | 45.9 | 85 | 100 |

- **Family history and sex of affected**
  In this study: 5 (5.9%) of the study sample said yes there is family history of related condition are siblings, 4 out of 5 (80%) mentioned bilirubin and only 1 (20%) said G6PD deficiency. While almost 80 (94.1%) of the study sample agree with the option NO (family history of related condition). Regarding the gender of affected siblings 2 out of 5 (40%) were male and 3 out of 5 (60%) were female. According to family history of dark urine (Jaundice) only 1 out of 5 (20.0%) said yes, 4 out of 5 (80.0%) mentioned that there is no family history of dark urine. (Table & figure : 3)

| Table & figure (3): Distribution of the study sample according to family history and sex of affected |
|---------------------------------|---------|---------|---------|
| Items | Yes | No | Total |
| Family history of related condition is siblings | NO % | NO % | NO % |
| 5 | 5.9% | 80 | 94.1% | 85 | 100 |
| If yes what? | Bilirubin | G6pd deficiency | Total |
| NO % | No % | NO % |
| 4 | 80% | 1 | 20% | 5 | 100 |
| Sex of affected siblings | Male | Female | Total |
| NO % | NO % | NO % |
| 2 | 40% | 3 | 60% | 5 | 100 |
| Family history of dark urine (Jaundice) | Yes | No | Total |
| NO % | NO % | NO % |
| 1 | 20.0% | 4 | 80.0% | 5 | 100 |

**Figure 3:**
- Deficient male 4.70%
- Deficient female 1.20%
- Normal 94.10%

- **Gender:**
  Gender of babies in the study samples the results showed that: 49 (57.6%) of them were male and 36 (42.4%) female. (Table & figure : 4)

| Table & figure (4): Distribution of the study sample according to gender of baby: |
|---------------------------------|---------|---------|---------|
| Sex of Baby | Male | Female | Total |
| No % | No % | No % |
| 49 | 57.6% | 36 | 42.4% | 85 | 100 |
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G6PD Result:
- **Qualitative Methaemoglobin Reduction Test:**
  Regarding screening results (qualitative methaemoglobin reduction test) this results showed that 80 (94.1%) of the study sample showed normal screening test, 4 (4.7%) showed deficient male and only 1 (1.2%) showed female(Table & figure 5).

<table>
<thead>
<tr>
<th>Screening results (qualitative methaemoglobin reduction test)</th>
<th>Normal</th>
<th>Deficient male</th>
<th>Deficient female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No (%)</td>
<td>80</td>
<td>4</td>
<td>1</td>
<td>85</td>
</tr>
<tr>
<td>%</td>
<td>94.1%</td>
<td>4.7%</td>
<td>1.2%</td>
<td>100%</td>
</tr>
</tbody>
</table>

* Table & figure (5): Distribution of the study sample according to screening results (qualitative):

- **Quantitative Assay of the Enzyme:**
  The deficient male in this study reading (25.2 – 26.0 – 44.2 and 46.3) Mu/10⁹ cell and 1 deficient female (66.6)Mu/10⁹ cell by spectrophotometer(Table & figure 6).

* Table & figure (6): Distribution of the study sample according to screening results quantitative assay of the enzyme:

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<table>
<thead>
<tr>
<th>Deficient male</th>
<th>Deficient female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>25.2</td>
</tr>
<tr>
<td></td>
<td>26.0</td>
</tr>
<tr>
<td>4</td>
<td>44.2</td>
</tr>
<tr>
<td></td>
<td>46.3</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>66.6</td>
</tr>
</tbody>
</table>

Figure 6: Residence of deficient group

Correlation:
- **Residence of Deficient Group:**
  According to residence show that all the deficient group in the study from rural area,(Table 7).
  
<table>
<thead>
<tr>
<th>Residence of deficient group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rural</td>
</tr>
<tr>
<td>urban 0%</td>
</tr>
<tr>
<td>; rural 100%</td>
</tr>
</tbody>
</table>

Table (7): Residence of deficient group

- **Family History in Deficient Group:**
  Table & figure (8): Show that (20%) of deficient group with family history and (80%) with no family history is sibling.
  
<table>
<thead>
<tr>
<th>Present of family history in deficient group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>20%</td>
</tr>
<tr>
<td>no</td>
</tr>
<tr>
<td>80%</td>
</tr>
</tbody>
</table>

Figure 8:

- **Family History of Dark Urine in Deficient Group:**
  According to family history of dark urine in this study(20%) of deficient group with family history of dark urine and (80%) with no family history of dark urine (Table & figure 9).
VI. DISCUSSION:

This results showed that 67 (78.8%) of study sample from rural area, while only 18 (21.2%) of them from urban area (Table & figure - 1). Regarding consanguinity of parents the results revealed that 21 (24.7%) of the subjects showed 1st degree relative, 25 (29.4%) 2nd degree relative while less than half 39 (45.9%) of the study sample showed no relation between the parents (Table 2). Also this results illustrate that only 5 (5.9%) of the study sample said yes there are family history of related condition in siblings, 4 out of 5 (80%) mentioned bilirubin and only 1 (20%) said G6PD deficiency. While almost 80 (94.1%) of the study sample agree with the option No (family history of related condition). Regarding the gender of affected siblings 2 out of 5 (40%) were male and 3 out of 5 (60%) were female. According to family history of dark urine (Jaundice) only 1 out of 5 (20.0%) said yes, 4 out of 5 (80.0%) mentioned that there is no family history of dark urine Table & figure : 3). Gender of babies in the study samples the results showed that: 49 (57.6%) of them were male and 36 (42.4%) female (Table & figure : 4). Regarding screening results (qualitative methaemoglobine reduction test) this results showed that 80 (94.1%) of the study sample showed normal screening test, 4 (4.7%) showed deficient male and only 1 (1.2%) showed female (table & figure- 5).Table & figure (6) show that the deficient male is homozygote deficient initial from 25.2Mu/10⁹ cell (low quantitative to 46.3 Mu/10⁹ cell high quantitative. But the female is heterozygote deficient quantitative read 66.6 Mu/10⁹ cell according to the normal reference of quantitative (80-180) Mu/10⁹ cell because screening qualitative interpreted between twocolour (normal colour blood and deficient colour blood). According to the residence in this study there were strong relation between the deficient group and rural(100%) (P 0.000), table 7, table & figure 8- 9). In this study the incidence of G6PD deficiency in Wad Medani Obstetric Gynecology teaching hospital was 4.7% in male population and 1.2% in female. This was in agreement with similar study done by Ramin Iranpor in Isfahan, Iran, which found that the incidence of G6PD deficiency in 2501 newborn examined by methaemoglobin reduction test in cord blood specimen was 5.1% in male population and 1% in female population (Ramin, et al, 2008). The study was in agreement with another study done in Hong Kong in Chinese patient, where 4.8% of male were found to be G6PD deficient (Auw, et al, 2009). Another study was similar with this study. That study was conducted in the special care neonatal unit of Bankura Sammilani Medical college, of the total 176 neonates, 13.63% had G6PD deficiency, most are male (Manik, et al, 2012). Another study was in agreement to this study; done by Michael Kaplan, of the total eight hundred and six new born screened for G6PD deficiency, 30.2% of the boys and 10.4% of the girls had sever G6PD deficiency (Michal, et al, 1994). The low incidence of G6PD deficiency in this study could be due to the small size of the sample. However, it was impractical and difficult to collect all specimens available at time of delivery particularly night, in addition to the logistical difficulties concerning collection.

VII. CONCLUSION AND RECOMENDATION

Conclusion
- G6PD deficiency is distributed in different rural among Sudanese populations with a percentage 4.7% among male newborns and 1.2% among female newborn in this study in comparison with urban populations (0%).
- There were strong relation between the deficient group and rural areas (P. value: 0.000). With that who was family history in 20% of the deficient group.
- Although the methaemoglobin reduction test (Motulsky dye discoloration test). Takes longer time than the florescent test. Its advantages include the fact that it is extremely cheap , easy and that the only equipment required is a water bath.

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Recomendation

- Neonates should be tested for G6PD deficiency if they have a family history of haemolysis or are of a particular ethnic or geographic origin, or if the presence of neonatal jaundice suggests the possibility of the disorder.
- The methaemoglobin reduction screening test which is specific, easy and cheap when compared to fluorescence spot test. Is recommended in the laboratory routine work.
- When clinical and haematological findings raise the suspicion of G6PD deficiency, the disorder should be confirmed by quantitative spectrophotometric measurement of red blood cell enzyme activity.
- Education programmes for susceptible families with G6PD deficiency newborns are highly recommended.
- Our data suggests that neonatal screening for G6PD deficiency is a useful test for preventing and early treatment of complication associated with it.
- New studies among a certain ethnically Sudanese population group should be performed to determine the prevalence or incidence of G6PD deficient enzyme by using other procedure e.g. fluorescence spot screening test. Which is sensitive and easy to perform.
- Electrophoresis and molecular techniques, should be introduced for detection of different variants.

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