ABSTRACT

Background: G6PD deficiency is the most common gene mutation in the world and the numerous mutations have been classified by the WHO.

Objective: is to estimate the prevalence of G6PD enzyme deficiency among ≤5 years old children attending Pediatric Hospital in center of Diyala province, Iraq, and to see whether screening for G6PD enzyme deficiency is worthy or not.

Study setting, design and sample size: A hospital based observational cross-sectional study consisting of 1500 children (820 boys and 680 girls), in children aged 5 years and below who attended Al-Batool Obstetrics and Pediatric Teaching Hospital in Baqubah city which is the center of Diyala province, Iraq during period of 7 months; from December 1st 2018 to June 30th 2019. Blood samples were collected and analyzed for Hemoglobin (Hb) level, and G6PD enzyme activity assay.

Results: out of 1500 children tested for enzyme activity only 20 children (1.33%) had low enzyme activity.

Conclusion: The prevalence of G6PD enzyme deficiency is low, so screening for the enzyme deficiency is not warranted at least in Diyala province.

Keywords: Screening, G6PD, children
Introduction:
G6PD deficiency is the most common gene mutation in the world [1]. The degree of G6PD deficiency varies from negligible to severe [2]. With a single allele, G6PD activity levels in males are either normal or deficient [3-7]. G6PD enzyme maintains reduced glutathione that defend against oxidative damage in RBCs, A lack of G6PD makes the RBCs fragile to oxidative stresses causing hemolysis. Severe cases in neonates can develop into kernicterus [8,9]. In 1989, (WHO) working group recommended that “whenever possible, neonatal screening should be performed in populations where G6PD deficiency is common (i.e., where it affects >3-5% of males) [10]. It is quite prevalent in many middle east countries including Iraq [11-13]. Data regarding its prevalence are available from different parts of Iraq [14,15]. The highest frequency (65%) of G6PD deficiency has been reported from eastern Saudi Arabia [16,17-19]. The prevalence in Oman is 25% in male and 10% in female; [16, 20, 21]. The frequency in Iraq is ranging from 6 to 13 % [22-25] while it ranges from 8%-25% in UAE [16, 26, 27]. In Kuwait its prevalence is 20.4% [28]. The low prevalence’s (< 5%) were found in Syria, Egypt, Libya, Lebanon, Tunisia & Algeria [16,29,30,23,31-35]. In India, the prevalence rate varied from 0 to 27% [36,37]. By 1988 around 400 types of G6PD were recognized with the most prevalent mutations in people from the Mediterranean, the west of Africa and Southeast Asia. [38-42]. Asians are more deficient than Africans, and peoples of the Mediterranean have the most severe form. [38]. Because of the clinical importance of the problem we decided to estimate the prevalence of G6PD enzyme deficiency among children aged 5 years and below in Diyala province and see whether screening of G6PD deficiency is recommended as a routine test as well as to see if there is a correlation between Hb level, blood group and Rh and residence of children to G6PD deficiency.

Patients and Methods:
A hospital based observational cross-sectional study consisting of 1500 children (820 males and 680 female), aged ≤ 5 years who attended Al Batool Obstetrics and Children Teaching Hospital in Baqubah city, Diyala, Iraq, which serves population of around 1.75 million indwelling Diyala province, during the period from December 1st 2018 to June 30th 2019.

Data of patients
A brief clinical data including age, gender, place of residence, blood group and Rh, family background including number of siblings, and any affected member in the family; history of consanguinity as well as history of past illnesses especially neonatal jaundice was recorded from all the participants.

Laboratory work
Blood samples were collected in K3-EDTA tubes from each child. All the samples were analyzed for Hemoglobin (Hb) and G6PD activity. The materials that were used, EDTA blood 4 ml, sodium nitrite 0.1 ml (store at 2-8) and methylene blue 0.05 ml (store below 25 ℃). Then two tubes named tube A & tube B, in each tube ,0.05 ml of sodium nitrite and 2 ml of EDTA blood were put and just in tube A added 0.05 ml of methylene blue, then both tubes were put in water bath at (37) for 3 hours, tube B used as a control and should always be brown. The results depended on the color of tube A, red color means no G6PD deficiency, brown color (like tube B) means deficiency of G6PD and between red and brown color means intermittent expression of G6PD deficiency. The test applied in this study was described in an article by Owa and Osanyintuy [43]

Exclusion criteria any child known to has G6PD deficiency and children above five years old.
**Ethical consent.** The research protocol and the questionnaires were conducted according to principles of the Declaration of Helsinki, as well as reviewed and approved by Ethics Research Committee of the College of medicine, Diyala university. Verbal consents were also taken from the parents and caregivers of children involved in the study.

**Statistical analysis**

The X² (Chi-squared) test method used to test theories on the differences between the percentages, a level of significance of α=0.05 was applied to test, the statistics software used to process the data analysis were the (SPSS version 22 and Excel 2013).

**Results:** A low frequency (1.33%, 20/1500) of G6PD enzyme deficiency was detected in this study. In males, it was 17 cases (85%), while in females it was 3 (15%).

**Table 1:** showed the sociodemographic characteristics of children with G6PD deficiency in studied sample. The age predominance was 25-36 months old. The male to female ratio was 5.6/1 with a significant difference between genders (p<0.05). Seventy percent of them had history on neonatal jaundice.

**Table 2** showed the relationship of Hb level to G6PD deficiency among positively screened children. Fifty-five percent had Hb level above 11gm/dL, with no significant statistical difference P value 0.65.

**Table 3** showed the relationship of blood group and Rh to G6PD deficiency among positively screened children. The blood group O⁺ve constituted 35% of positively screened children, P value 0.001.

**Discussion:**

G6PD enzyme deficiency usually asymptomatic, but they can develop severe jaundice during the neonatal period and acute hemolytic anemia when they consume fava beans or when they are exposed to infection or medications.

**Table 1: The sociodemographic characteristics of Twenty children with G6PD deficiency**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
<th>No.</th>
<th>%</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mo.)</td>
<td>≤12</td>
<td>2</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13-24</td>
<td>4</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25-36</td>
<td>7</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>37-48</td>
<td>4</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>49-60</td>
<td>3</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>17</td>
<td>85</td>
<td>0.001***</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>3</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td>Rural</td>
<td>8</td>
<td>40</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>Urban</td>
<td>12</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Consanguinity</td>
<td>No</td>
<td>15</td>
<td>75</td>
<td>0.001***</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>5</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Family History of G6PDdeficiency</td>
<td>No</td>
<td>16</td>
<td>80</td>
<td>0.001***</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>4</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>History of neonatal jaundice</td>
<td>Yes</td>
<td>14</td>
<td>70</td>
<td>0.001***</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>6</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

Previous studies had shown a higher prevalence of the disorder among the general population indicating the need for screening as a routine test. Studies from different parts of Iraq revealed variable results from Baghdad (6.1-12.4%) at the center of country and 15.3% from Basra city and 6% in Sulymania [11,13,14,15,42]. It had higher prevalence in nearby countries like Saudi Arabia, Oman, UAE, and Kuwait [16,20,27,28], while it was lower in Ethiopia, Iran and Egypt (1.4%,3.2% and 4.3%) respectively [4, 41,44].

**Table 2: Hemoglobin level distribution among positively screened children**

<table>
<thead>
<tr>
<th>Hemoglobin level (gm/dL)</th>
<th>No.</th>
<th>%</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below 11</td>
<td>9</td>
<td>45</td>
<td>0.65</td>
</tr>
<tr>
<td>Above 11</td>
<td>11</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
In this study of 1500 screened children only (20) 1.33% turned to have deficient enzyme, this may be attributed to many reasons such as, the geographical area and gene frequency may be low in comparison to others localities, the size of the sample which was relatively small, as well as the method of detection of enzyme deficiency was qualitative one. Family history is negative in 16/20 (80%) which is not essential for diseases occurrence as it is x-linked recessive disorder. Neonatal jaundice was noted in14/20 (70%) of positively screened children due to hemolysis as it is essential for stabilization of RBCs when exposed to oxidizing agent at any time of life. Hemoglobin level was normal in 11 /20 (55%) and 9/20 (45%) had Hb below 11gm/dl since most patients with G6PD deficiency are asymptomatic and their Hb are kept at normal range if no other complicating events which reduce Hb level, however 45% of them had Hb level below normal which may be due to other etiologies than G6PD deficiency, or subclinical or spontaneous hemolysis owing to severity of enzyme deficiency as noticed by a Chinese study [23], in this study we noticed 7/20 (35%) had association with blood group O**, which is statistically significant in comparison to other blood groups which is on contrary to what was found by Saha N. el al [2], this may be due to different sample size and different methodology used by previous study.

Conclusions: The routine Screening of G6PD enzyme deficiency in Diyala province is not justified, but this need to be confirmed with further larger studies. More than half of positively screened children had normal Hb level.

Recommendation: According to results of this study, screening of G6PD enzyme deficiency may be unjustified as a routine test. Future larger studies to ascertain the results of this study as well as the detection of the phenotyping of the enzyme deficiency in Diyala province is warranted.

Acknowledgments Many thanks to all medical staff of Al Batool teaching hospital, Baqubah, Diyala, for providing help and assistance and for laboratory of Al Batool teaching hospital for their collaboration, during the study and samples collection, and also for all patients who were cooperative with us.

References:

Table 3: Distribution of ABO and Rh blood groups of the G6PD deficiency in twenty children

<table>
<thead>
<tr>
<th>ABO Blood group</th>
<th>Rh</th>
<th>No.</th>
<th>%</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Positive</td>
<td>4</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Positive</td>
<td>4</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>Positive</td>
<td>2</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>Positive</td>
<td>7</td>
<td>35</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


[38] Chan TK. Glucose-6-phosphate dehydrogenase deficiency: A review glucose-6-phosphate dehydrogenase. University of Hong Kong; 2013.


