Glucose-6-Phosphate Dehydrogenase Activity in Jordanian Female Children with Acute Hemolytic Crises

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Abstract

Objective: the study objective is to determine the mode of inheritance in Glucose-6-Phosphate Dehydrogenase (G6PD)- deficient female children who have a history of acute hemolytic crises. Also, it aims to study the relationship between the type of inheritance and the severity of hemolysis, and to determine the factors incriminated in provoking hemolytic crises on them.

Materials and Methods: The study included 40 female children with confirmed diagnosis of G6PD deficiency who were admitted to Princess Rahma Teaching Hospital with acute hemolytic crisis between June 2002 and April 2005. G6PD enzyme levels were studied in their mothers and in thirty-seven fathers to determine the mode of inheritance. In addition, 45 unaffected volunteers were evaluated as a control group.

Results: The results showed that 65 % were heterozygous females and 35 % were homozygous for G6PD deficiency. The mean G6PD level for homozygous females was 4.79 ± 2.67 mU/10^9 RBCs compared to 54.1 ± 23.2 mU/10^9 RBCs in heterozygous females. In 92.5% of children there was a history of fava beans ingestion before crisis. The mean time for appearance of symptoms after ingestion of fava beans was 10.57 ± 4.99 hours compared to 23.77 ± 5.23 hours in heterozygous females, and the duration of hemolytic crises was higher in homozygous females: 78 ± 12.2 hours compared to 53 ± 10 hours in heterozygous females. Two breast-feed infants gave history of fava beans ingestion by their mothers before crisis.

Conclusion: This study demonstrated that females with G6PD activity level of less than 10% are usually homozygous females with rapid onset of symptoms and longer duration of hemolytic crisis following exposure to triggering factors, especially uncooked green beans, whose metabolites may be excreted in breast milk.

Keywords: Glucose-6-Phospahte Dehydrogenase, Acute Hemolytic Crises, inheritance.

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Introduction

G6PD deficiency is the most common known enzymopathy in humans. The defect is inherited as x-linked disorder, thus it is fully expressed in all the erythrocytes of affected homozygous males, while in females, G6PD deficiency may be either homozygous or heterozygous. In populations with high gene frequency and high rate of consanguineous marriages, such as Jordan, the rate of homozygous females could be high, and hemolysis could be as severe as in homozygous males. In theory, heterozygous females should be clinically normal, but as a matter of fact, there are reported cases of severe hemolytic crises in such patients, perhaps due to a variable inactivation of the x-chromosome in such females. [1-4]

The aim of this study was to determine the G6PD activity levels in female children that were admitted with acute hemolytic crises to Princess Rahma Teaching Hospital in the north of Jordan and to determine their type of inheritance and its relationship with the severity of their disease.

Materials and Methods

This prospective study included all female children with acute hemolytic anemia due to G6PD deficiency that were admitted to Princess Rahma Teaching Hospital between June 2002 and April 2005.

Acute hemolytic anemia was considered when there was sudden pallor, drop of hemoglobin, and other features such as jaundice, abdominal pain, and change in the color of urine and presence of hemoglobinuria. All cases were investigated during hemolytic crises for their level of Hb, complete blood counts, hemoglobinuria, G6PD level, liver function, kidney function and Coomb's test. An enquiry was made about the time duration from exposure to oxidants to the appearance of symptoms, duration of hemolytic crises, type of food ingested, medication before crisis, family history of favism and parental consanguinity.

Forty girls aged between 9 months and 10 years with confirmed history of acute hemolysis due to G6PD deficiency were included in the study. After obtaining informed consent from their parents, blood samples were collected from the affected girls (more than 3 months after hemolytic crisis), from their 40 mothers, 37 available fathers, and from 45 controls, of which 15 were adult males, 15 adult females and 15 were girls of < 10 years of age. The samples were examined for G6PD enzyme activity; and then followed the blood films preparation for reticulocytes. For fathers who were not available, the type of inheritance of their daughters was determined by polymerase chain reaction for the presence of one or two mutant genes.

Quantitative G6PD assay was performed using commercial G6PD kits (Randox Laboratories Ltd, United Kingdom) by measuring G6PD activity levels in erythrocytes, and was expressed as $mU/10^9$ RBCs. Complete blood counts and other hematological parameters were measured by using a fully automated Coulter counter. Reticulocyte counts were estimated by brilliant cresyl blue stained slides.

The normal G6PD enzyme activity was estimated according to the measured enzyme activity in age-matched control group. The cut-off point of G6PD deficiency was considered as < 2 Standard Deviation (SD) below the mean of the enzyme activity of the controls.

Statistical analyses were preformed using Minitab version 11 (Minitab for Windows). Data were expressed as means ± SD. Two-tailed t-test was used for comparison. Differences between independent sample populations were considered significant if the p value was found to be less than 0.05.

The study was approved by the Ethics Committee of the Jordan University of Science and Technology and was supported by the Deanship of Research. Informed consents were obtained from all parents and controls.

Results

The study included 40 female children with acute hemolytic crisis due to G6PD deficiency, in addition to 77 parents and 45 healthy volunteers.

The mean values of G6PD activity in control children was found to be 123 ± 15 mU/10^9 RBCs, and in the control male and female adults it was 119 ± 14 mU/10^9 RBCs. Thus, the cut-off point of G6PD deficiency in affected females was considered any value less than 93 mU/10^9 RBCs and the cut-off point of G6PD deficiency for parents was considered as any value < 91 mU/10^9 RBCs.

As expected, the mean value of G6PD in the patients was found significantly lower than that of the control group, while the other hematological parameters were found to be the same in both groups, as summarized in Table (1). The results of liver and renal function tests were normal while Coombs tests were negative.

The level of G6PD was found deficient in 21 (57%) available fathers, with a mean value of 9 ± 11 mU/10^9 RBCs, and was found within the normal in 16. On the other hand, 32 (80%) of the mothers had low G6PD level with mean value of 57.8 ± mU/10^9 RBCs, while 8 mothers were considered normal.

In this study, 26 patients (65%) were heterozygous and 14 (35 %) were homozygous for G6PD deficiency depending on the presence of low level of G6PD in either one or both of the parents, and according to results of gene mutation in the 3 girls with unavailable fathers (2 homozygous and 1 heterozygous).

A comparison was done between heterozygous and homozygous patients with regard to the level of G6PD, Hb during crisis and at steady state, the time between exposure and appearance of hemolysis symptoms, the duration of crisis as calculated from the duration of hospital stay, reticulocytic count during steady state, MCV, MCH and MCHC.

It was found that the mean Hb level during crisis was lower in homozygous compared to that of heterozygous patients, while no significant difference was found between Hb level in the steady state, MCV, MCH and MCHC and reticulocytes count as shown in Table (2).

The precipitating factors incriminated in provoking hemolytic crises were ingestion of fresh green fava beans in 26 patients, cooked green fava in 8 patients, and in 3 patients' ingestion of boiled dry fava beans. Two infants aged 9 and 11 months, who were exclusively breastfed, had a history of green fava ingestion by their mothers. In one female there was no history of fava beans ingestion but she had a history of fever and antibiotic administration, while none of the other patients had history of fever or drug administration. In homozygous patients, the duration between ingestion of fava beans and the first appearance of symptoms of hemolytic crisis was shorter after fresh green fava ingestion (9.3 hours ± 4.3) compared to cocked (14 hours ± 3.4) and dry beans (18 hours ± 1). Two of heterozygous patients had clinical evidence of chronic hemolytic anemia with a mean Hb level of 7.3 g/dl, and reticulocytes of 10 % and mean G6PD activity level of 49.5 mU/10^9 RBCs.

Discussion

G6PD deficiency is an X-linked recessive disorder, with expected severe enzyme deficiency in homozygous males and females. Heterozygous females appear to be less affected and often have normal or moderate-to-severe decrease in enzymatic activity. [5, 6]

The reported prevalence of G6PD deficiency in the north of Jordan was 5.5%. [7] Moreover, it was found that 17 % of children admitted to Princess Rahma Teaching Hospital with acute hemolytic anemia due to G6PD deficiency were females. [8]

In the present study, we found that 35 % of the affected 40 girls were homozygous and 65 % were heterozygous. This result was similar to that reported by Madanat et al. who found that 3 of 8 Jordanian female patients were homozygous and

5 were heterozygous. [9] The high rate of consanguineous marriages in Jordan may explain this high incidence of homozygous cases. This is clearly demonstrated in the present study, as 9 (64%) of homozygous patients had parents that were first or second cousins while only 3 (11%) of heterozygous parents were closely related ($p < 0.001$). Similar findings were reported by Al-Ali et al. [10] who found that most of females patients with G6PD deficiency were homozygous in Oman, where the rate of consanguineous marriages is high. [10] on the other hand, Abdulrazzaq et al. [11] reported that despite the high level of consanguinity in the United Arab Emirates, only 10% of females were homozygous for G6PD. This difference between the findings of his study and the present study may be explained by the difference between the two study populations, as he studies females with a positive family history of G6PD deficiency not symptomatic G6PD deficient patients like our group.

A significant difference was found between G6PD activity level and the severity of hemolytic crisis between homozygous and heterozygous cases. All the homozygous patients had an enzyme activity level of less than 10 mU/10^9 RBCs, while the heterozygous patients had levels that ranged between 12 to 90 mU/10^9 RBCs. This difference could be explained by the inheritance of two defective genes in homozygous patients, while in heterozygous cases the enzyme activity depended on preferential expression of the G6PD-deficient gene and X-inactivation of the normal gene during embryogenesis. [1-6, 11] The severity of crisis was found more obvious in homozygous compared to heterozygous patients, which was evident by the rapid appearance of the hemolytic symptoms after exposure to fava beans, lower Hb level at admission and longer duration of hemolytic crisis. This statistically significant difference in the severity of hemolytic crisis between homozygous and heterozygous patients is probably related to the lower enzyme activity in those patients. The present study supports the finding of Russo et al. [3] who reported that the severity of hemolytic crises is proportionate to the amount of enzyme in the RBCs of homozygous and heterozygous females.

Severe hemolytic crisis in patients with G6PD deficiency after ingestion of fava beans is frequent among the population of the Mediterranean countries due to the widespread human ingestion of this type of beans. [12, 13] Fava beans, particularly fresh green broad beans, are rich in two biologically inactive β-glycosides compounds, vicine and convicine, which hydrolyze enzymatically to form aglycones, divicine and isouramil. These compounds are powerful oxidants which rapidly oxidize glutathione and cause hemolysis in G6PD-deficient red cells. [13, 14] In this study, 92.5% of patients with G6PD deficiency had their hemolytic crisis after ingestion of fava beans.

The crisis was triggered by green uncooked fava bean in 70% with the earliest onset of appearance and longest duration of hemolytic symptoms followed by cooked green beans (15%), then dry beans (7.5%). The present study is consistent with what was reported by Madanat et al. [5] who found that the main triggering factor for hemolysis was ingestion of fresh uncooked fava beans. Two infants (5%), one homozygous for G6PD and aged 9 months, the other heterozygous and aged 11 months, had their crisis after being breastfeed after the ingestion of cooked fava beans by their mothers. This was similar to the finding of Kaplan et al., [15] who reported favism in a breastfed newborn infant whose mother had ingested fava beans, and to the report of Corchia et al. [16] which described favism in a female newborn infant whose mother ingested fava beans before delivery. Therefore, it is important to advice mothers of G6PD infants to avoid ingestion of fava beans if they are breastfeeders, particularly during the neonatal period to prevent the occurrence of kernicterus.

Beutler et al. [17] reported that a small number of G6PD deficient individuals have rare mutations with very longstanding hemolysis in the absence of oxidant stress.

Two (5%) of our heterozygous patients had clinical evidence of chronic hemolytic anemia with a mean Hb level of 7.3 g/dl, and reticulocytes of 10 % and mean G6PD activity level of 49.5 mU/10^9 RBCs. However, they did not have any exposure to oxidant stress such as fava beans, drugs or infections during the period of the study.

The present study demonstrated that females with G6PD activity level that is less than 10 % usually have rapid onset of symptoms after exposure to triggering factors, and they have longer duration of hemolytic crisis, particularly, after the ingestion of uncooked green beans which may be excreted by breast milk.

Table (1): The mean G6PD activity level and hematological parameters for affected females and controls.

Variable	*Affected females. No. 40 Mean (SD)*	*Controls No.15 Mean (SD)*	*P-value**
G6PD (mU/10^9 RBCs)	*38 (2)*	*123 (15)*	*<0.0001*
Hb (g/dl)	*11.8 (1)*	*12.3 (1)*	*0.067*
RBCs (10^6/mm^3)	*4.3 (0.4)*	*4.7 (0.6)*	*0.255*
MCV (fl)	*82 (5)*	*82 (4)*	*0.744*
MCH (pg)	*27.3 (1.7)*	*28 (2)*	*0.474*
MCHC	*33.5 (0.8)*	*34 (2)*	*0.106*

**** P-value significant at < 0.05***

Table (2): Comparison between homozygous and heterozygous patients.

Variables	*Homozygous. No.14 Mean (SD)*	*Heterozygous.No.26 Mean (SD)*	*P-value**
Hb (g/dl) level in the steady state **	*11.6(0.76)*	*11.8 (1.58)*	*0.48*
Hb during crisis (g/dl)	*5.0 (0.75)*	*6.9 (1.35)*	*0.0001*
G6PD (mU/10^9 RBCs) In the steady state**	*4.7 (2.67)*	*54.1 (23.2)*	*<0.0001*
MCV (fl)	*82.3 (2.5)*	*81.4 (6.01)*	*0.613*
MCH (pg)	*27.6 (0.8)*	*27.2 (1.92)*	*0.476*
MCHC	*33.6 (0.7)*	*33.5 (0.85)*	*0.685*
% of Reticulocyte count	*1.25 (0.2)*	*1.7 (2.5)*	*0.48*
Duration of the first symptoms after ingestion of fava beans (hours)	*10.5 (4.9)*	*23.7 (5.2)*	*< 0.0001*
Duration of crises (hours)	*78 (12.2)*	*53 (10)*	*< 0.0001*

****P-value significant at < 0.05***
*****more than 3 months after the crisis***

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نشاط إنزيم نازعة هيدروجين الجلوكوز 6 فوسفات (G6PD) في إناث الاطفال الأردنيين المتأثرات بالازمات الانحلالية

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الملخص

الهدف: هدفت هذه الدراسة إلى قياس مستوى نشاط نازعة هيدروجين الجلوكوز6 فوسفات (G6PD) في كل من الاطفال الإناث المتأثّرات بالأزمات الإنحلالية، والكشف عن نوع الوراثة في اكتساب النقص في الانزيم لتلك الحالات اعتماداً على قياس مستوى نشاط إنزيم في أبائهن، والكشف عن العوامل الأكثر سبباً في حدوث أزماتهن الانحلالية.

عينة البحث: شمل البحث 40 طفلة من اللواتي أدخلن إلى مستشفى الأميرة رحمة باربد وهن متأثرات بالأزمات الانحلالية الحادة ما بين حزيران 2002 ونيسان 2005 ، و 40 مِن أمهاتهن، و37 عينة مِن آبائهن. ثلاثة آباء رفضوا إعطاء عينة الدم ووراثة النقص في الإنزيم في بناتهم أُكد بعمل سلسلة تفاعل إنزيم البلمرة (PCR) للتقرير إذا ما كن ثنائي الأليل homozygous أو أحادي الأليل heterozygous. كما شملت الدراسة 45 من متبرعين غيرمصابين للمقارنة.

النتائج: أوضحت نتائج هذه الدراسة أن 65 % ورثن نقص الإنزيم من أحد الأبوين واعتبرن أحادية الأليل لنقص الإنزيم و أن 35 % من المصابات ورثن نقص الإنزيم من الأب والأم واعتبرن ثنائي الأليل، ووجد أن متوسطَ نشاط الإنزيم لإناث ثنائي الأليل هو(4.79 ± 2.67 مل وحدة/ 109 كرية دم حمراء) وهو أقل من حالات احادية الأليل (54.1 ± 23.2 مل وحدة/109 كرية دم حمراء) ووجد أن متوسط الوقت لظهور إنحلال الدم (10.57 ± 4.99 ساعة) لإناث ثنائية الأليل وهوأقل من ذلك لإناث أحادية الأليل (23.77± 5.25 ساعة)، وإن المدة المتوسطةَ لاستمرار الأزمات الإنحلالية هي (78 ± 12.2 ساعة) لثنائي الأليل وهي أعلى من ذلك لإناث أحادية الأليل (53 ± 10 ساعة). وأن أكل الفول الأخضرالطازج غيرالمطبوخ هو العامل الأكثرشيوعاً والذي يسبب الأزمات الانحلالية الحادة. كما وجد ان كل الإناث ثنائية الأليل و73 % من إناث أحادي الأليل كان عندهن تاريخ عائلي إيجابي. ووجد أن طفلتين (5 %) لإناث أحادي الأليل عندهن فقرالدم الإنحلالي المزمن وطفلتين أصبن بالأزمة الإنحلالية الحادة عندما رضعن من أمهاتهن اللواتي أكلن فولاً مطبوخاً اثناء إرضاعهن.

الخلاصة: اثبتت الدراسة أن الإناث المتأثرات بنقص الإنزيم لمستوى أقل من 10% هن غالبا ثنائي الأليل، مع سرعة ظهور إنحلال الدم فيهن بعد التعرض للعوامل المسببة خاصة بعد أكل الفول الأخضر الطازج غير المطبوخ والذي من الممكن ان يفرز مع حليب الام.

الكلمات الدالة: إنزيم نازعة هيدروجين الجلوكوز 6 فوسفات، الأزمة الإنحلالية، الوراثة.