original research report

Predictors of severe hemolysis in patients with glucose-6-phosphate dehydrogenase deficiency following exposure to oxidant stresses

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BACKGROUND AND OBJECTIVES: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a genetic enzymatic disorder that affects millions of people worldwide, and is a major health problem in Jordan. We studied factors that may predict severe hemolysis in children with G6PD deficiency.

METHODS: We reviewed the records of patients with low G6PD activity admitted to a teaching hospital between 1996 to 2007. We collected demographic data, details of sign and symptoms, history and type of fava bean ingestion, blood and Rh group, history of neonatal jaundice, history and type of drug use, abdominal pain at admission and the results of tests for hemoglobin, white blood cells (WBC), and hepatic function. We classified patients into mild and severe groups based on hemoglobin levels at admission.

RESULTS: Of 428 children with G6PD deficiency, 79 (18%) were severe cases and 349 (82%) patients with mild disease. There were no statistically significant differences in most factors between the two groups. Factors that achieved statistical significance for severe hemolysis included younger age ($P<.05$), male gender ($P<.05$), higher alkaline phosphatase (ALP) ($P<.05$), presence of fever at admission ($P<.01$), presence of vomiting during the attack ($P=.006$), and a negative family history for G6PD deficiency ($P=.005$).

CONCLUSIONS: Severe hemolysis can be predicted during hemolytic episodes in children with low G6PD by young age, male gender, a negative family history of G6PD deficiency, the presence of fever and vomiting and a high ALP.

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a genetic enzymatic disorder affects millions of people worldwide.[1,2] About 7.5% of the population of the world are carriers for G6PD deficiency, ranging from 0.1% in Japan and some European areas to 35% in Africa, Southern Europe, the Middle East, and Southeast Asia.[3-5] The condition is usually asymptomatic, but ingestion of or contact with fava beans, drugs or chemicals, or bacterial or viral infections may cause acute hemolytic anemia.[6,7] As G6PD deficiency is an X-linked genetic abnormality the clinical manifestations of G6PD deficiency are much more common in male homozygotes than in females heterozygotes. This genetic abnormality is associated with a range of clinical conditions. Some subjects are asymptomatic, whereas others suffer from neonatal jaundice, acute hemolytic anemia or rarely severe non-spherocytic hemolytic anemia.[8,9] Patients with hemolysis are always G6PD deficient, but not all G6PD deficient individuals develop hemolysis following exposure to oxidant stresses, presumably because other factors are involved.[7,10,11] G6PD deficiency is still a major health problem in Jordan. This study was conducted to assess for the presence of any factors in the medical history or results of investigations that might be associated with severe hemolysis in children with G6PD deficiency treated at Princess Rahma Hospital over an 11-year period.

METHODS

We reviewed the medical charts of patients with G6PD

deficiencies who presented with acute intravascular hemolysis and who were admitted to Princess Rahma Children's Hospital in Irbid, Jordan between January 1996 and December 2007. We included all patients with a clinical diagnosis of acute hemolysis and a confirmed diagnosis of G6PD deficiency by measuring the level G6PD enzyme activity at admission. Some individuals with severe hemolysis may have had normal red cells at the time of hemolysis, only to revert to a deficiency state a few weeks later. Patients with suspected G6PD deficiency, but with a normal test, were invited for a repeat test 6 weeks later to confirm the diagnosis. If they did not have G6PD deficiency, they were excluded from the study. The criteria for clinical diagnosis was when the child presented with sudden pallor, jaundice and had a dark or cola-colored urine, an elevated reticulocyte count, indirect bilirubin, and lactate dehydrogenase. Ethical committee approval was obtained from the Princess Rahma Children's Hospital Research Committee.

To assess the predictors of severity, we divided the patients into mild and severe groups based only on their hemoglobin at the time of admission. If it was less than ≤5 g/L, it was regarded as severe hemolysis and if more it was regarded as mild hemolysis. A special data collection sheet was developed for the sake of the study. Differences in clinical and biological characteristics between the two groups were collected for the two study groups and analyzed using the Fisher exact test, the chi-square test, and the t test wherever appropriate. Information about potential predictors of hemolysis were collected including patient age at admission, gender, white blood cell count (WBC) at admission, alanine aminotransferase (ALT) level at admission, aspartate aminotransferase (AST) level at admission, presence of fever (defined as temperature ≥38°C orally) at admission, fava bean ingestion or exposure (fresh vs. cooked vs. dried), presence of vomiting during the attack, presence of a positive family history with G6PD deficiency (defined as history of hemolysis in the family secondary to G6PD deficiency), blood group (O vs. A. vs B vs. AB), Rh positive vs. negative, history of neonatal jaundice, drug use (defined as any drug that induced hemolysis in G6PD deficiency), prior infection (defined by clinical presentation or confirmation by laboratory investigation), and presence of abdominal pain at admission. A *P* value of less than .05 was considered statistically significant. The Statistical Package for Social Sciences (SPSS, version 15) software was used to analyze data.

RESULTS

Of 428 children with G6PD deficiency, 79 (18%) were severe cases and 349 (82%) patients with mild disease (Table 1). Severe cases were significantly younger than mild cases. Pallor of abrupt onset and passage of cola-colored urine were universal presenting symptoms for all the cases. Incriminating factors responsible for hemolysis included ingestion of fava beans (n=399), drug intake (n=30) and infection (n=75), with more than one predisposing condition existing in some children. A marked elevation in serum bilirubin, coinciding with intravascular hemolysis, was a feature in all the 428 children with G6PD deficiency.

There was no statistically significant difference in the mean white blood cells count (WBC) at admission, ALT level at admission, AST level at admission, type of fava bean ingestion, blood group, Rh positivity, history of neonatal jaundice, drug use (defined as any drug that is known to induce hemolysis in G6PD deficiency), prior infection (defined by clinical presentation or confirmation by laboratory investigation), and presence of abdominal pain at admission between the two groups. Factors associated with significantly higher hemolysis compared to mild included younger age, male gender, higher alkaline phosphatase enzyme level, presence of fever at admission, presence of vomiting during the attack, and a negative family history for G6PD deficiency (Table 1).

DISCUSSION

G6PD deficiency is still the most common of all clinically significant enzyme defects, not only in hematology, but in human biology as a whole. A variety of drugs, foods and infections cause hemolytic anemia in persons with the deficiency, and nonhematologic sequelae have been claimed as well.[6-9] Using classical biochemical techniques, an enormous diversity of mutations causing G6PD deficiency have been documented in hundreds of publications.[8-11]

This is the first report on predisposing factors for severe hemolysis in G6PD deficiency. Our results revealed that the frequency of the enzyme deficiency in males (76%) was higher than in females (24%). A higher incidence in males than females was also reported in many studies.[12,13] The incidence of the severe form of the enzyme deficiency in females is considerably lower, with most of the cases being of the mild form of the enzyme deficiency.[12,13] Because G6PD deficiency is a sex-linked disorder, it shows full expression in heterozygous males and homozygous females. Heterozygous females exhibit variations in expression depending on the degree of inactivation. Thus, heterozygous females may have undetectable, intermediate, and normal enzyme levels in their erythrocytes. Our results showed

Table 1. Baseline characteristics and results of the analysis among the two study groups.

Number	Cases with mild hemolysis n=349	Cases with severe hemolysis n=79
Age (years)[a]		
Mean, standard deviation	4.2 (2.9)	2.9 (1.9)
Range	0.2-13	1-13
Sex (n, %)[a]		
Male	255 (73)	71 (90)
Female	94 (27)	8 (10)
Alkaline phosphatase enzyme level (U/L) (mean, standard deviation)[a]	169 (18)	252 (25)
Presence of fever[b]	48%	73%
Presence of vomiting[c]	20%	34%
Negative family history of G6PD deficiency[d]	37%	55%
Presence of abdominal pain	16.3%	16.5%
Ingestion or exposure to favabeans		
Fresh	45%	31%
Cooked	42%	52%
Dried	13%	17%
Ingestion of drugs	4%	2%
Rh		
Positive	92.4%	92.8%
Negative	6.6%	6.8%
Blood group		
O	36.5%	36.5%
A	42.1%	32.4%
B	16.1%	21.6%
AB	5.3%	9.5%
History of neonatal jaundice	41%	31%
Mean WBCs $\times 10^9$	14.1	19.3
Mean ALT enzyme level(U/L)	47	28
Mean AST enzyme level(U/L)	38	37

[a] $P<.05$, [b] $P<.01$, [c] $P=.006$ [d] $P=.005$ severe vs. mild hemolysis groups. WBC: white blood cell; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

that male gender is a predisposing factor for severe hemolysis, which probably reflects the fact that those females with so-called mild deficiency were heterozygous for the deficient gene.

This is the first report to show an association between age at presentation and severity of hemolysis. Younger patients are probably exposed to more than one incriminating factor. Infection-induced hemolysis is a common cause of clinically significant hemolysis. Many different types of infections may cause significant hemolysis in the G6PD deficient patient.[14-19] The mechanism by which this occurs is not clear, but it has been suggested that leukocytes damage erythrocytes in their environment by discharging active oxygen species during phagocytosis, or perhaps nitric oxide might also play such a role.[14,20] Our results revealed fever as a significant predictor of severe hemolysis. Moreover, Mean WBC count was higher in the severe cases, but the difference was not statistically significant.

Gastric upset, abdominal pain, and back pain are associated with severe hemolysis due to nitric oxide depletion or hemoglobin urea. Our results showed vomiting as a significant predictor of severe hemolysis, but no difference in the frequency of abdominal pain between the two groups. Our data showed that a negative family history for hemolysis secondary to G6PD deficiency is a significant predictor factor for severe hemolysis probably due to attitude and knowledge of their families. Patients with a positive family history for hemolysis secondary to G6PD deficiency probably institute preventive measures for exposure to oxidant stresses or they seek medical care earlier following exposure to oxidant stresses.

A limitation of the study is that it was retrospective and another problem is that males and females were pooled. No biochemical test can accurately identify all female heterozygotes. Heterozygosity may have resulted in a more mild form of hemolysis, but this is not accounted for in the study. Many studies of G6PD deficiency have, for this reason, included males only. Increased hemolysis is not always a major factor in the development of neonatal jaundice.[21] Moreover, even in the absence of all known triggers, jaundice can occur. An alternative factor influencing the development of jaundice may be defective liver enzymes.[22-25] Our data showed that liver enzymes were affected more in the severe cases. Severe cases had a higher alkaline phosphatase enzyme level than mild cases. Although mean ALT was higher in the severe cases the difference was not statistically significant.

In summary, this is the first study to demonstrate factors that predict severe hemolysis in patients with G6PD deficiency. We identified some factors that predict severe hemolysis among G6PD deficient individuals. Cord blood screening of G6PD level is indicated in a high-risk population. Once G6PD-deficiency is confirmed, close monitoring, health

education regarding triggers, and proper follow-up for hemolysis can prevent severe hemolysis, thus decreasing morbidity and mortality in patients with G6PD deficiency.

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