

# Association of Serum Zinc Level with Severity of Acute Hemolysis in Iranian Children with Glucose-6-Phosphate Dehydrogenase Deficiency

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## Abstract

**Background:** Glucose-6-phosphate dehydrogenase (G6PD) deficiency increases the vulnerability of red blood cells to oxidative damage. In this study, we aimed to evaluate the association of serum zinc level with the severity of G6PD deficiency in children with acute hemolysis.

**Methods:** This cross sectional study was performed on 40 children, admitted to hospital due to acute hemolysis of G6PD deficiency. Hemoglobin level, number of transfusions, severity of hemoglobinuria, duration of acute hemolysis, and severity of hemoglobin drop were recorded; moreover, serum zinc level was monitored. Statistical analyses were performed using SPSS version 18.

**Results:** The serum concentration of zinc was higher in male patients compared to females ( $P = 0.044$ ), while hemoglobin level upon admission was lower in male patients ( $P = 0.013$ ). The serum level of zinc had no association with hemoglobin level (0.333), number of transfusions ( $P = 0.604$ ), severity of hemoglobinuria ( $P = 0.569$ ), duration of acute hemolysis ( $P = 0.908$ ), and severity of hemoglobin drop ( $P = 0.932$ ).

**Conclusions:** This study revealed that serum zinc concentration was not associated with the severity of acute hemolysis in G6PD-deficient children.

**Keywords:** Children, Zinc, Acute Hemolysis, Glucose-6-Phosphate Dehydrogenase Deficiency

## 1. Background

Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme involved in the pentose phosphate pathway, which catalyzes the reduction of nicotinamide adenine dinucleotide phosphate (NADP) to NADPH and protects the cells against oxidative damage (1). G6PD deficiency increases the vulnerability of red blood cells (RBC) to oxidative damage (2). G6PD gene is located on the distal long arm of X chromosome (2); accordingly, males are more affected than females (3).

G6PD deficiency is prevalent throughout Africa, Asia, and the Middle East (4). Clinical presentations of this disease may include acute hemolytic anemia, chronic hemolytic anemia, and neonatal hyperbilirubinemia; the disease may be also asymptomatic (2). Acute hemolysis may occur due to infection, consumption of fava beans, and drugs (5). It may be accompanied by abdominal pain, jaundice, organomegaly, and hemoglobinuria (6). The main treatment of G6PD deficiency is avoidance of oxidative stressors; however, anemia may be so severe to warrant blood transfusions (7).

Some antioxidants, such as vitamin E and selenium, have been studied in G6PD deficiency treatment with no definite effects (5, 7). Studies have evaluated the level of antioxidants in acute hemolysis and their supplements in preventing G6PD hemolysis (8-12). Zinc is one of these antioxidants, which has been examined in few studies (9-12); nonetheless, some controversial findings have been reported. Considering the conflicting data, we aimed to evaluate the association of serum zinc level with the severity of acute hemolysis due to G6PD deficiency.

## 2. Methods

We evaluated 40 children, referred to the emergency pediatric ward of Shiraz University of Medical Sciences due to acute hemolysis of G6PD deficiency. The inclusion criteria were as follows: 1) age below 18 years; 2) confirmed G6PD deficiency based on quantitative spectrophotometric analysis in infancy; and 3) diagnosis of acute hemolysis symptoms (e.g., hemoglobinuria and hemoglobin drop). On the other hand, the exclusion criteria were as follows: 1) other

causes of hemolysis including Coombs-positive hemolysis; and 2) other systemic hepatobiliary diseases, renal problems, vasculitis, and chronic anemia.

For the examinations, 2 mL of peripheral blood was collected from children within 2 hours of admission due to acute hemolysis. The samples were centrifuged and serum was preserved at  $-70^{\circ}\text{C}$ . The serum level of zinc was analyzed via atomic absorption spectrophotometry at Shiraz endocrinology and metabolism research center. The present study was approved by the ethics committee of Shiraz University of Medical Sciences. All the parents signed the informed consent forms.

Data analyses were performed using SPSS version 18 (Chicago, IL, USA). Data are presented as mean  $\pm$  SD. Normality of data distribution was evaluated by Kolmogorov-Smirnov test. Student t test and Chi square test were used to compare quantitative and qualitative variables, respectively. P value less than 0.05 was considered statistically significant.

### 3. Results

In the present study, 40 children with known G6PD deficiency and acute hemolysis were enrolled. In total, 24 (60%) patients were male. Table 1 summarizes the general characteristics of the patients. As the findings revealed, 30 (75%) patients did not receive any zinc supplements. The serum concentration of zinc was higher in male patients than females ( $P = 0.044$ ), while hemoglobin level upon admission was lower in males ( $P = 0.013$ ). Other factors did not differ significantly (Table 1).

For evaluating the severity of acute hemolysis, duration of hemolysis, hemoglobin concentration at admission, severity of hemoglobinuria, and number of transfusions for treatment were recorded. Table 2 summarizes the severity of acute hemolysis in our patients. In total, 27.5% of the patients had severe acute hemolysis. Upon admission, 17.5% of the patients had a hemoglobin level below 5 g/dL, which was more prevalent in males (25% vs. 6.3%;  $P = 0.009$ ). Duration of hemolysis was  $2.5 \pm 2.1$  days and was not significantly different among male and female patients.

Overall, 32.5% of the patients had hemoglobinuria more than 2 plus, although the difference between males and females was insignificant. Based on the findings, 10% (3 males and 1 female) of the patients needed transfusion more than twice to be hemodynamically stable. The serum level of zinc was not associated with age ( $P = 0.264$ ), serum glutamic oxaloacetic transaminase (SGOT) ( $P = 0.722$ ), serum glutamic-pyruvic transaminase (SGPT) ( $P = 0.342$ ), alkaline phosphatase ( $P = 0.383$ ), hemoglobin level (0.333), number of transfusions ( $P = 0.604$ ), severity

of hemoglobinuria ( $P = 0.569$ ), duration of acute hemolysis ( $P = 0.908$ ), and severity of hemoglobin drop ( $P = 0.932$ ).

### 4. Discussion

The present study revealed that acute hemolysis due to G6PD deficiency was more severe in males than females. Zinc level was higher in boys than girls; however, serum zinc level was not associated with the severity of hemolysis. Overall, acute hemolysis in G6PD-deficient children occurs after exposure to an oxidative stressor; nevertheless, it does not persist in spite of continuing stressor. This is due to the fact that older erythrocytes with more enzyme deficiency first undergo hemolysis (2), and then, younger erythrocytes with higher enzyme activity sustain oxidative damage without hemolysis (13).

A previous study revealed that zinc deficiency could cause oxidative damage to proteins, lipids, and DNA in rat testes (14). Also, Gomez et al. showed that chronic zinc deficiency had prooxidative effects on the lungs. Another study showed that higher levels of serum copper and magnesium in G6PD-deficient male adults play an important role in RBC resistance to plasmodium falciparum, whereas serum zinc and calcium did not have major effects (9). Vitamin E scavenges free radicals and is involved in restoration of normal serum concentrations of copper and zinc in G6PD-deficient hemolysis (10). Similar to our study, Korunanithy et al. showed that serum and RBC zinc content did not significantly change in G6PD-deficient patients during hemolysis (11).

This study is the first evaluation of the association of serum zinc level with the severity of acute hemolysis in G6PD deficiency. However, severity of G6PD deficiency was not quantitatively measured, which is a shortcoming of this study.

#### 4.1. Conclusion

This study revealed that serum zinc concentration was not associated with the severity of acute hemolysis in G6PD-deficient children. Further studies are needed to evaluate this association.

**Table 1.** General Characteristics of G6PD-Deficient Patients<sup>a</sup>

Variables	Total, 40	Male, 24 (60)	Female, 16 (40)	P Value
Age	4.62 ± 2.57	4.23 ± 2.14	5.21 ± 3.09	0.260
Duration of acute hemolysis, days	2.52 ± 2.17	2.25 ± 1.07	3.19 ± 2.93	0.942
<b>History of zinc consumption</b>				0.263
No	30 (75.0)	16 (66.7)	14 (87.5)	
Yes	10 (25.0)	8 (33.3)	2 (12.5)	
Zinc level, µg/mL	104.34 ± 27.59	112.50 ± 32.30	92.10 ± 10.66	0.044
SGOT, IU/L	49.20 ± 27.82	47.95 ± 17.53	51.07 ± 39.18	0.751
SGPT, IU/L	17.22 ± 12.96	15.91 ± 11.11	19.18 ± 15.51	0.0.299
Alkaline phosphatase, IU/L	511.28 ± 230.58	520.80 ± 89.84	497.00 ± 95.16	0.72
Hb, g/dL	6.91 ± 1.75	6.50 ± 1.78	7.53 ± 1.58	0.0.013

<sup>a</sup>Values are expressed as No. (%).**Table 2.** Information Related to the Severity of Acute Hemolysis in G6PD-Deficient Patients<sup>a</sup>

Variables	Total, 40		Male, 24 (60)	Female, 16 (40)	P Value
Duration of acute hemolysis, days	2.52 ± 2.17		2.25 ± 1.07	3.19 ± 2.93	0.942
Hemoglobin level on admission	< 5	7 (17.5)	6 (25.0)	1 (6.3)	0.009
	5.1 - 7	12 (30.0)	10 (41.7)	2 (12.5)	
	> 7	21 (52.5)	8 (33.3)	13 (81.3)	
Hemoglobinuria (+)	0	15 (37.5)	10 (41.7)	5 (31.3)	0.146
	1	4 (10.0)	2 (8.3)	2 (12.5)	
	2	8 (20.0)	2 (8.3)	6 (37.5)	
	3	6 (15.0)	4 (16.7)	2 (12.5)	
	4	7 (17.5)	6 (25.0)	1 (6.3)	
Number of needed transfusions	0	1 (2.5)	0 (0)	1 (6.3)	0.094
	1	35 (87.5)	21 (87.5)	14 (87.5)	
	2	3 (7.5)	3 (12.5)	0 (0)	
	3.00	1 (2.5)	0 (0)	1 (6.3)	

<sup>a</sup>Values are expressed as No. (%).**Table 3.** Information Related to the Severity of Acute Hemolysis in G6PD-Deficient Patients

Variables	Severe Hemolysis	Mild Hemolysis
Age	5.1 ± 3	4.4 ± 2.4
Zinc level, µg/mL	114 ± 40	100.4 ± 20.2
SGOT, IU/L	48.3 ± 22.9	49.5 ± 29.8
SGPT, IU/L	15.7 ± 7.2	17.7 ± 14.6
Hb, g/dL	5.9 ± 2	7.2 ± 1.5
Sex, male, female	8, 3	16, 13
<b>Hemoglobinuria (+)</b>		
0	0	15
1	1	3
2	3	5
3	3	3
4	4	3
<b>Number of needed transfusions</b>		
0	0	1
1	7	28
2	3	0
3.00	1	0

## Footnotes

**Conflicts of Interest:** The authors declare no conflicts of interest.

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