

# Antioxidant vitamins and glucose-6-phosphate dehydrogenase deficiency in full-term neonates

## Antioxidative Vitamine und Glucose-6-Phosphat-Dehydrogenase-Mangel bei ausgereiften Neugeborenen

### Abstract

**Objective:** The mechanism by which glucose-6-phosphate dehydrogenase (G6PD) deficiency causes neonatal hyperbilirubinemia is not completely understood. However, the genetic disorder G6PD deficiency predisposes red blood cells to oxidative stress. The aim of this study was to establish the relationship between plasma antioxidant vitamin (E and C) levels and the development of hyperbilirubinemia in full-term neonates with deficient G6PD.

**Methods:** A total of 196 live birth neonates of healthy mothers were included in this study. Twelve of them were deficient in G6PD. In addition to demographic data, serum total bilirubin, hemoglobin, hematocrit, and vitamin E and C levels were measured on the first day after birth.

**Results:** Neonates with G6PD deficiency (n=7) who did not develop hyperbilirubinemia (mean serum bilirubin level of  $70.8 \pm 23 \mu\text{mol/l}$ , median 71.8) and neonates with G6PD deficiency (n=4) who developed hyperbilirubinemia (mean serum bilirubin level of  $226.7 \pm 79 \mu\text{mol/l}$ , median 233.4) on the first day of life had similar gestational weights and age. The second group, however, had lower hemoglobin and hematocrit as well as plasma vitamin C and E levels. None of these results showed significant difference.

**Conclusion:** The results of the present study indicate that red blood cell hemolysis as a result of inadequate antioxidants system in G6PD-deficient neonates is not the only contributing factor for hyperbilirubinemia.

**Keywords:** bilirubin, vitamin C, vitamin E, glucose-6-phosphate dehydrogenase deficiency, G6PD deficiency

### Zusammenfassung

**Ziel der Studie:** Der Mechanismus, durch den ein Mangel an Glucose-6-Phosphat-Dehydrogenase (G6PD) bei Neugeborenen eine Hyperbilirubinämie erzeugt, ist noch nicht vollständig erforscht. Allerdings schafft der genetische Defekt eines G6PD-Mangels eine Veranlagung der roten Blutzellen zu oxidativem Stress. Das Ziel der vorliegenden Arbeit war es, die Beziehung zwischen den Konzentrationen antioxidativer Vitamine (Vitamin E und C) im Plasma und der Entstehung der Hyperbilirubinämie bei ausgereiften Neugeborenen mit G6PD-Mangel festzustellen.

**Methoden:** 196 Neugeborene von gesunden Müttern wurden in diese Studie eingeschlossen. 12 dieser Neugeborenen hatten einen G6PD-Mangel. Neben den demografischen Daten wurden am 1. Tag nach der Geburt die Konzentrationen von Gesamtbilirubin, Hämoglobin, Hämato-krit sowie Vitamin E und C bestimmt.

**Ergebnisse:** Neugeborene mit einem G6PD-Mangel (n=7), die keine Hyperbilirubinämie entwickelt haben (mittlerer Serum-Bilirubin-Spiegel  $70,8 \pm 23 \mu\text{mol/l}$ , median  $71,8 \mu\text{mol/l}$ ), und Neugeborene mit einem G6PD-Mangel (n=4), die am 1. Lebensstag eine Hyperbilirubinämie entwickelt haben (mittlerer Serum-Bilirubin-Spiegel  $226,7 \pm 79 \mu\text{mol/l}$ , median  $233,4 \mu\text{mol/l}$ ), waren in Bezug auf Geburtsgewicht und

Khalid K. Abdul-Razzak<sup>1</sup>  
Enaam M. Almomany<sup>1</sup>  
Mohamad K. Nusier<sup>2</sup>  
Ahmad D. Obediat<sup>3</sup>  
Ahmad M. Salim<sup>4</sup>

1 Department of Clinical Pharmacy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan

2 Department of Biochemistry and Molecular Biology, Jordan University of Science and Technology, Irbid, Jordan

3 Department of Paediatrics, Princess Rahma Hospital, Irbid, Jordan

4 College of Applied Medical Science, Jordan University of Science and Technology, Irbid, Jordan

Schwangerschaftsalter vergleichbar. Die zweite Gruppe wies allerdings niedrigere Konzentrationen von Hämoglobin, Hämatokrit sowie Vitamin C und E auf. Aber keines dieser Messergebnisse zeigte eine signifikante Differenz.

**Schlussfolgerung:** Die Ergebnisse der vorliegenden Studie zeigen, dass Hyperbilirubinämie nicht allein durch Hämolyse als Folge eines unzureichenden Antioxidationsystems bei Neugeborenen mit G6PD-Mangel verursacht wird.

**Schlüsselwörter:** Bilirubin, Vitamin C, Vitamin E, Glucose-6-Phosphat-Dehydrogenase-Mangel, G6PD-Mangel

## Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is the first enzyme in the pentose phosphate pathway in red blood cells [1]. The main physiologic role of this pathway is to generate the reduced nicotinamide adenine dinucleotide phosphate (NADPH) necessary for protection of the cells against oxidative stress [2]. NADPH maintains glutathione in the reduced state when erythrocytes are subjected to an oxidative stress [3]. Low activities of G6PD render red blood cells susceptible to hemolysis under certain conditions such as ingestion of fava beans, certain drugs, and severe infections [1], [4].

G6PD deficiency is one of the most common human enzyme deficiencies, affecting more than 400 million people worldwide [5], [6]. Common clinical manifestations of G6PD-deficient subjects are neonatal jaundice and acute hemolytic anemia [7]. Most people with this disorder are male since the inherited deficiency is an X-linked enzymatic defect [7], [8]. G6PD deficiency is the most significant factor that contributes to severe neonatal hyperbilirubinemia and kernicterus [9], [10]. Distribution of the deficiency varies among different populations reflecting geographic and ethnic variations [6] with high prevalence in the Middle East [4].

Antioxidant vitamins (E and C) are considered one of the defense systems against reactive oxygen species [11]. Vitamin E is a potent antioxidant that effectively protects biological membranes against oxidative injury [12]. Supplementation of vitamin E to preterm infants with hyperbilirubinemia results in significant reduction in plasma bilirubin level which is suggested to be caused by a reduction in red cell hemolysis [13]. Previously, we have reported the association between plasma antioxidant vitamins (E and C) and hyperbilirubinemia in full-term neonates [14]. We attributed the significant hyperbilirubinemia to increased oxidative stress due to low levels of antioxidant vitamins and in turn erythrocytes haemolysis.

A limited number of studies on the role and status of vitamin E and C in neonate with G6PD deficiency with or without hyperbilirubinemia can be found in literature. Therefore, this study was undertaken to assess the status of plasma vitamin E and C and to establish the relationship between antioxidant vitamin levels and the development of hyperbilirubinemia in full-term neonates with deficient G6PD.

## Materials and methods

### Study protocol

This study was approved by The Review Committee for Research on Human at Jordan University of Science and Technology (JUST). The population of this study consisted of live birth neonates that consecutively delivered at Princess Rahma Hospital, Irbid-Jordan, for the period from March to July 2004. Both females and males were included in this study. In all cases gender, birth weight and gestational age were recorded.

### Laboratory analyses

All laboratory analyses were conducted on venous blood obtained into heparinized glass tubes from all infants included in this study on the day of birth as stated previously [14]. In brief, plasma total bilirubin level was measured using bilirubin analyzer BA III (Tokyo, Japan). Hemoglobin and hematocrit were measured using automated blood counter (Micros 60 OT, Montpellier, France). For detection of G6PD deficiency, a methemoglobin reduction test was used [15]. Plasma vitamin C level was measured by calorimetric method [16]. Plasma vitamin E level was measured by high performance liquid chromatography (HPLC) [17].

### Statistical analysis

Data was evaluated by Minitab Statistical Software release 14. Mann-Whitney test was used to compare the significance of median difference between the two groups. P values <0.05 were considered statistically significant.

## Results

During the study period, 196 neonates were studied, out of which 12 (6.1%) were deficient in G6PD enzyme: 11 full-term neonates (10 male and 1 female) and one premature male. Gestational age and birth weight of the two groups showed no significant differences.

The hemoglobin and hematocrit were lower in neonates with hyperbilirubinemia than in neonates without hyper-

**Table 1: Demographic data of the neonates studied**

Parameters	Gestational age (week)		Birth weight (kg)		Hemoglobin (g/l)		Hematocrit (%)	
	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)
G6PD-deficient neonates who did not develop hyperbilirubinemia (n=7)	38.4 (1.3)	38 (37–40)	2.8 (0.4)	2.8 (2.3–3.5)	182 (14)	182 (164–200)	58.7 (5.4)	58.9 (50–66)
G6PD-deficient neonates who developed hyperbilirubinemia (n=4)	39 (1.8)	39 (37–41)	3.2 (0.2)	3.1 (3–3.5)	158 (31)	157 (122–197)	50 (7.8)	48 (43–61)

n= number of neonates

**Table 2: Plasma vitamin E and C levels in the neonates studied**

Parameters	G6PD-deficient neonates who did not develop hyperbilirubinemia (n=7)		G6PD-deficient neonates who developed hyperbilirubinemia (n=4)		P value (95% CI)
	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)	
Vitamin C (µmol/l)	122.6 (33)	124.9 (58–153)	91.9 (16.5)	90.8 (74–114)	0.0108 –0.2997, 1.0997
Vitamin E (µmol/l)	10.9 (6)	9.5 (6–22)	7.1 (2)	7.4 (5–9)	0.138 (–0.500, 5.801)
Bilirubin (µmol/l)	70.8 (23)	71.8 (41–99)	223.7 (79)	233.4 (118–310)	0.0107 (–13.900, –2.698)

n= number of neonates

bilirubinemia, but the differences were not statistically significant (Table 1).

Similar results were found for plasma vitamin E and C levels. They were lower in neonates with hyperbilirubinemia than in neonates who did not develop hyperbilirubinemia, but the differences were not statistically significant (Table 2).

## Discussion

High bilirubin load due to erythrocyte hemolysis plays an important role in neonatal hyperbilirubinemia. The reasons are higher erythrocyte turnover, shorter life span and diminished capacity of neonatal erythrocytes to deal with oxidative stress as a result of decreased antioxidant defence system particularly in the preterm infants [18], [19], [20], [21]. Erythrocytes are prone to oxidative reactions due to relatively high oxygenation, hemoglobin, and plasma membrane rich in a polyunsaturated fatty acids [22]. The magnitude of hemolysis has been shown to be directly proportional to the amount of free radicals present that can be inhibited by antioxidant substances [23], [24]. The mechanism by which G6PD deficiency causes neonatal hyperbilirubinemia is not completely understood [25]. Red blood hemolysis may be observed [25] or found

to be associated [26] with jaundice in neonates with G6PD deficiency. The genetic disorder G6PD deficiency predisposes red blood cells to oxidative stress and hence, depending on the balance of oxidant species and antioxidants, this may lead to red cell hemolysis and hyperbilirubinemia [27]. Ojo et al. [28] observed that full-term neonates with highest plasma bilirubin levels had lowest plasma vitamin E values, especially in G6PD-deficient erythrocytes. Vitamin E is known to be a cellular antioxidant [22] that prevents membrane damage by inhibiting lipid peroxidation [11].

Vitamin C is a water soluble antioxidant and has also been found to suppress erythrocyte hemolysis induced by water-soluble radical initiator [29]. The synergistic effect between vitamin E and C in inhibition of lipid peroxidation has been reported previously [30].

In our study, neonates of both groups had similar gestational weights and age. Despite these similarities, hemoglobin, hematocrit as well as plasma antioxidant vitamins E and C levels were lower in neonates who developed hyperbilirubinemia. This finding explains the protective action of these vitamins against hemolytic anemia in G6PD-deficient neonates who developed hyperbilirubinemia. This is in agreement with Bizzarro et al. [31] who reported that hemolysis may be observed in neonates with G6PD deficiency and jaundice that exacer-

bated by oxidative stress [32]. Moreover, our findings indicate that red blood cell hemolysis due to the toxic effects of oxidants and the inadequate antioxidants system are not the only contributing factors for development of hyperbilirubinemia in G6PD-deficient neonates. This corresponds with Jalloh et al. [33] and Kaplan et al. [34], who found that hemolysis is not the main determinant of neonatal jaundice in G6PD-deficient babies. Hyperbilirubinemia in G6PD-deficient infants is also likely to occur secondary to inability of the liver to adequately conjugate and clear bilirubin as a result of a mutation of uridine diphosphoglucuronate glucuronosyltransferase-1 gene promoter leading to hyperbilirubinemia [25], [35]. Weng et al. [36] showed that G6PD-deficient neonates were at increased risk for hyperbilirubinemia and that G6PD activity of male newborns that developed hyperbilirubinemia was significantly lower than the neonates without hyperbilirubinemia. This points out the importance of knowing the degree of G6PD activity since this enzyme is responsible for the generation of NADPH, an important factor in the generation of reduced glutathione, which is an endogenous antioxidant affecting the balance between oxidants and antioxidants.

## Conclusion

In the present study, it was shown that the development of hyperbilirubinemia in G6PD-deficient neonates can not be attributed only to hemolysis of red blood cells as a result of inadequate antioxidants system.

## Notes

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## Conflict of interest

None declared.

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**Corresponding author:**

Khalid K. Abdul-Razzak, PhD  
 Department of Clinical Pharmacy, Jordan University of Science and Technology, Faculty of Pharmacy, PO Box 3030, Irbid-22110 Jordan, Tel.: 00962 2 7201000 Ext. 23536, Fax: 00962 2 7201075  
 kkalani@just.edu.jo

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