

Antioxidant vitamins and hyperbilirubinemia in neonates

Antioxidative Vitamine und Hyperbilirubinämie bei Neugeborenen

Abstract

Objective: Low antioxidant system may contribute to the severity of neonatal hyperbilirubinemia. The aim of this research was to explore the relationship between plasma vitamin E and C levels and the severity of hyperbilirubinemia in full-term neonates with normal glucose 6-phosphate dehydrogenase (G6PD) activities.

Methods: A total of 130 full-term healthy live birth neonates of healthy mothers with normal G6PD activity were included in this study. In addition to routine blood analysis, plasma total bilirubin, vitamin E and C levels and G6PD activity were measured on the first day of life. None of the neonates was ABO incompatible or anemic.

Results: Neonates who did not develop hyperbilirubinemia (n=119) had a mean plasma bilirubin level of 65 ± 24 $\mu\text{mol/l}$ (median 58.1), while neonates who developed significant hyperbilirubinemia (n=11) had a mean plasma bilirubin level of 238 ± 56 $\mu\text{mol/l}$ (median 246.2) on the first day of life. Mean plasma vitamin C levels of neonates who developed hyperbilirubinemia were significantly lower than those who did not develop hyperbilirubinemia (87 ± 22 $\mu\text{mol/l}$ (median 89.4) vs. 132 ± 36 $\mu\text{mol/l}$ (median 127.7), respectively, $P=0.0001$). Similar results were observed for plasma vitamin E levels in neonates who did or did not develop hyperbilirubinemia (7.5 ± 2 $\mu\text{mol/l}$ (median 6.3) vs. 10.4 ± 5 $\mu\text{mol/l}$ (median 9.1), respectively, $P=0.001$). Hemoglobin and hematocrit were significantly lower in neonates who developed hyperbilirubinemia ($P=0.0002$ and $P=0.0003$, respectively), although gestational age and birth weight for the two groups showed no significant difference.

Conclusion: The results of the present work indicate that low level of plasma vitamins C and E are associated with significant hyperbilirubinemia in full-term neonates.

Keywords: hyperbilirubinemia, vitamin C, vitamin E, newborn

Zusammenfassung

Zielsetzung: Ein vermindertes antioxidatives System kann zur Verstärkung der neonatalen Bilirubinämie führen. Das Ziel dieses Projektes war die Beziehung zwischen den Konzentrationen der Vitamine E und C im Plasma und die Höhe der Hyperbilirubinämie bei Neugeborenen mit normaler Glucose-6-phosphat-Dehydrogenase-Aktivität zu erforschen.

Methoden: 130 am regelrechten Termin geborene gesunde Neugeborene mit normaler Glucose-6-phosphat-Dehydrogenase-Aktivität von gesunden Müttern wurden in diese Studie einbezogen. Neben den Routineanalysen im Blut wurde Gesamtbilirubin, Vitamin E und C im Plasma sowie die Glucose-6-phosphat-Dehydrogenase-Aktivität am 1. Lebenstag bestimmt. Keines der Neugeborenen hatte eine ABO-Inkompatibilität oder eine Anämie.

Ergebnisse: Neugeborene, die keine Hyperbilirubinämie entwickelt hatten (n=119), hatten am 1. Lebenstag einen mittleren Plasma-Bilirubinspiegel von 65 ± 24 $\mu\text{mol/l}$ (Median 58,1 $\mu\text{mol/l}$), während Neugeborene mit einer signifikant erhöhten Hyperbilirubinämie (n=11) mittlere Plasma-Bilirubinkonzentrationen von 238 ± 56 $\mu\text{mol/l}$ (Median 246,2 $\mu\text{mol/l}$) aufwiesen.

Khalid K. Abdul-Razzak¹
Mohamad K. Nusier²
Ahmad D. Obediat³
Ahmad M. Salim⁴

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

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

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

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

Der mittlere Vitamin C-Spiegel im Plasma von Neugeborenen, die eine Hyperbilirubinämie entwickelt hatten, war mit 87 ± 22 $\mu\text{mol/l}$ (Median $89,4$ $\mu\text{mol/l}$) signifikant niedriger als der von Neugeborenen, die keine Hyperbilirubinämie aufwiesen (132 ± 36 $\mu\text{mol/l}$, Median= $127,7$ $\mu\text{mol/l}$). Diese Unterschiede waren statistisch signifikant ($P=0,001$).

Ähnliche Ergebnisse wurden bei den Vitamin E-Spiegeln bei Neugeborenen beobachtet, die eine Hyperbilirubinämie entwickelt bzw. nicht entwickelt hatten: Die Vitamin E-Spiegel lagen bei $7,5 \pm 2$ $\mu\text{mol/l}$ (Median $6,3$ $\mu\text{mol/l}$) bzw. bei $10,4 \pm 5$ $\mu\text{mol/l}$ (Median $9,1$ $\mu\text{mol/l}$).

Hämoglobinkonzentrationen und Hämatokritwerte waren bei den Neugeborenen, die eine Hyperbilirubinämie entwickelt hatten, signifikant niedriger, obgleich die Schwangerschaftsdauer und die Geburtsgewichte der beiden Gruppen keine Unterschiede zeigten.

Schlussfolgerung: Die Ergebnisse der vorliegenden Studie zeigen, dass bei ausgereiften Neugeborenen niedrige Konzentrationen von Vitamin C und Vitamin E im Plasma mit einer signifikant erhöhten Hyperbilirubinämie korreliert sind.

Schlüsselwörter: Hyperbilirubinämie, Vitamin C, Vitamin E, Neugeborene

Introduction

Hyperbilirubinemia is a common problem in neonatal period worldwide [1] and is a main cause of readmission to hospitals [2]. Increased production of bilirubin, immature hepatic uptake and conjugation processes, and increased enterohepatic circulation of bilirubin are the most common factors that contribute to the development of hyperbilirubinemia in neonates [3], [4]. The combination of glucose-6-phosphate dehydrogenase deficiency, the most common enzyme deficiency known and Gilbert's syndrome, which is characterized by decreased activity of bilirubin-conjugating enzymes, increase the possibility of severe neonatal hyperbilirubinemia [5]. Higher bilirubin load due to erythrocyte hemolysis play an important role in neonatal hyperbilirubinemia due to higher erythrocyte turnover and shorter life span (80 days compared with the adult 120 days) [6], [7], [8].

Normal newborn erythrocytes are relatively sensitive to oxidative damage in comparison with those of adults [9]. This is due to diminished capacity of neonatal erythrocytes to deal with oxidative stress as a result of decreased antioxidant defense system particularly in the preterm infants [10]. The body possesses an efficient antioxidant defense system against oxidative damage. A series of enzymes, including catalase, superoxide dismutase, and glutathion peroxidase and nonenzymatic antioxidants such as glutathione, vitamins E and C [11]. Bilirubin, in particular unconjugated bilirubin which is regarded as a toxic metabolic waste, also was shown to have antioxidant role [12]. Also bilirubin has been shown to display some toxicity towards erythrocytes [13].

The relationship between preterm neonatal hyperbilirubinemia and reduced antioxidants vitamins and enzymes has been reported [14], [15]. However, there are contradictory reports regarding plasma vitamin C level and the development of hyperbilirubinemia in preterm neonates.

Ballin et al. [16] described Heinz body hemolytic anemia in premature infants as a result of high vitamin C supplementation. Such finding had not been supported by the work of Doyle et al. [17]. Also Bass et al. [18] found the administration of vitamin C to preterm neonates to be safe and not associated with hemolysis.

Low level of vitamin E, the potent antioxidant that effectively protects biological membranes against oxidative injury [19], was also attributed to a significant hyperbilirubinemia in full-term neonates [20], while the role of vitamin C in the development of hyperbilirubinemia in full-term neonates has not been reported.

The aim of the present study was to investigate the relationship between vitamin E and C levels and the severity of hyperbilirubinemia in full-term neonates with normal glucose 6-phosphate dehydrogenase (G6PD) activity.

Material and methods

Patients

The study group consisted of full-term healthy live birth neonates of healthy mothers that occurred at Princes Rahma Hospital, Irbid-Jordan for the period from March to July 2004. The University Review Committee for Research on Human approved this study. Healthy neonates and mothers were discharged within 24 hours for vaginal births and within 48-72 hours for cesarean births, as the hospital follows an early discharge protocol. In all cases gender, birth weight, gestation period, and delivery route were recorded. A total of 130 full-term neonates with normal G6PD activity and gestational period >37 week were included in this study. One hundred nineteen of them had serum bilirubin levels <102 $\mu\text{mol/l}$ with a mean of 65 ± 24 $\mu\text{mol/l}$ (median 58.1) on the first day of life. None of these were readmitted to the hospital for significant hyperbilirubinemia. Yet 11 full-term neonates had serum bilirubin levels ≥ 102 $\mu\text{mol/l}$ with a mean of

238±56 µmol/l (median 246.2) on the first day of life and all of them received either phototherapy treatment or blood exchange. None of the neonates included in this study was ABO incompatible or anemic. Therefore, the significant hyperbilirubinemia was not related to hemolytic disease.

Blood samples

Venous blood was obtained into two heparnized glass tubes from all infants included in this study on the day of birth. One tube was used for routine blood analysis. The other tube was placed immediately on ice and used for vitamin E, C and G6PD activity measurements. For vitamin C determination, samples were stored at 4 °C for no more than 30 minutes before the plasma was separated and vitamin C was stabilized by metaphosphoric acid. Plasma vitamin C level and G6PD activity were measured within 3 hours after blood collection. For vitamin E measurements, 100 µl of plasma was stored at -20 °C until analysis.

Laboratory technique:

Plasma total bilirubin levels were measured using bilirubin analyzer BA III (Tokyo, Japan), while hemoglobin and hematocrit were measured using automated blood counter (Micros 60 OT, France). Methaemoglobin reduction test was used for detection of G6PD deficiency [21]. Plasma vitamin C levels were measured by calorimetric method [22], while vitamin E levels were measured by HPLC as described by George et al. [23].

Statistical analysis

Data was evaluated by Minitab release 14 statistical software. 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

The result of this study revealed that mean plasma vitamin C level of the first day of life of full-term neonates who did develop hyperbilirubinemia was significantly lower than that of full-term neonates who did not develop hyperbilirubinemia (Table 1).

Similar significant difference has been noticed for plasma vitamin E levels in neonates who did or did not develop hyperbilirubinemia. The clinical characteristics of the neonates are shown in Table 2. Hemoglobin and hematocrit were significantly lower in neonates who developed hyperbilirubinemia, while gestational age and birth weight of the two groups showed no significant differences.

Discussion

Erythrocyte hemolysis plays an important role in the development of hyperbilirubinemia in neonates [24]. Erythrocytes from neonates are susceptible to peroxide hemolysis due to continuous exposure to high concentration of oxygen and high polyunsaturated rich plasma membrane [19], [25]. Higher levels of lipid peroxidation markers were found in plasma and in the erythrocytes at birth, especially in preterm neonates [26], [27]. This finding was a reflex of low levels of antioxidant vitamins and/or low activities of antioxidant enzymes [28] and the reduction in plasma bilirubin was associated with an increase in plasma antioxidant capacity. Decrease in oxidative stress in preterm neonates was also reported [29]. Vitamin E and C are potent antioxidants at the cellular level, thus play an important role in the protection of cells against oxidative damage and hemolysis. Therefore, the level of vitamins E and C may be associated with the development of hyperbilirubinemia in neonates.

In this study, plasma vitamin E and C concentrations were measured during the first day of life in 119 neonates who did not develop hyperbilirubinemia and 11 neonates who did develop significant hyperbilirubinemia. Alpay et al. [30] used a serum bilirubin level of 120 µmol/l in predicting the development of significant hyperbilirubinemia in all healthy full-term neonates. Nearly all full-term neonates with serum bilirubin level of <120 µmol/l in the first day of life did not develop hyperbilirubinemia. Such observation has been supported by the prospective cohort study of Stevenson et al. [31].

Our study clearly demonstrates the association between low levels of vitamin E and C and neonatal hyperbilirubinemia. These results were in agreement to those obtained by Ojo et al. [20] who observed significant low plasma vitamin E level in full-term neonates associated with significant increase in bilirubin level and red blood cell hemolysis.

Our finding is also similar to what has been previously reported for the association between antioxidant plasma vitamins A, E, and C as well as erythrocyte antioxidant enzymes and hyperbilirubinemia in preterm neonates [14]. Lower level of antioxidant defense system was found in preterm infants with hyperbilirubinemia (bilirubin 256 µmol/l) than in those of the preterm infants with no jaundice (bilirubin 68-102 µmol/l). Also the administration of vitamin E to premature infants who are known to have low vitamin E concentration [32], [33] reduces red cell hemolysis and significantly decreases bilirubin level on the first week of life [33].

On the other hand, Bracci et al. [15] showed that erythrocyte antioxidant enzymes activities were significantly lower in neonates with peak bilirubin (>214 µmol/l) than in less jaundiced neonates on the 4th day of life. Although they did not measure the level of antioxidant vitamins, indirectly it can be concluded that antioxidant vitamins are low since they are dietary micronutrients [34].

Vitamin C is a water soluble antioxidant, also found to suppress erythrocyte hemolysis induced by water-soluble

Table 1: Plasma vitamin E, C and bilirubin levels in full-term neonates with or without hyperbilirubinemia

Parameters	Neonates without hyperbilirubinemia (n=119)		Neonates with hyperbilirubinemia (n=11)		P value (95% CI)
	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)	
Vitamin C (µmol/l)	132 (36)	127.7 (56-250)	87 (22)	89.4 (62-124)	0.0001 (0.4500,1.0501)
Vitamin E (µmol/l)	10.4 (5)	9.1 (4-40)	7.5 (2)	6.3 (5-14.8)	0.001 (0.500,1.700)
Bilirubin (µmol/l)	65 (24)	58.1 (15-100)	238 (56)	246.2 (143-306)	0.0001 (-12.400,-8.370)

Table 2: Some clinical data of full-term neonates with or without hyperbilirubinemia

Parameters	Gestational Age (Week)		Birth Weight (g)		Hemoglobin (g/l)		Hematocrit (Percent)	
	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)
Neonates without hyperbilirubinemia (n=119)	38.8 (1.2)	39 (37-43)	3327 (435)	3200 (2000-4500)	170 (17)	169* (126-219)	52 (5)	52** (36-94)
Neonates with hyperbilirubinemia (n=11)	38.3 (0.8)	38.5 (37-39)	3109 (317)	3200 (2400-3400)	152 (14)	149 (109-185)	47 (5)	46.2 (33-60)

*P=0.0002 (95% CI: 1.100, 3.000)

**P=0.0003 (95% CI: 3.301, 9.701)

radical initiator, but vitamin E which functions as antioxidant within membrane was found to be more effective in suppressing the hemolysis [35].

The synergistic effect between vitamin E and C in inhibition of lipid peroxidation has been reported [36]. Vitamin C helps in regeneration of vitamin E as vitamin E is oxidized by free radicals in the lipid bilayer [37], [38].

In conclusion, the results of the present work indicate that low levels of plasma vitamins E and C are associated with significant hyperbilirubinemia in full-term neonates due to increased oxidative stress and intern red blood hemolysis.

Notes

Conflicts of interest

None declared.

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Corresponding author:

Khalid K. Abdul-Razzak

Department of Clinical Pharmacy, Jordan University of Science and Technology, Faculty of Pharmacy, P.O Box 3030. Irbid 22110, Jordan, Tel.: 962-2-7201000 Ext. 23536, Fax: 962-2-7201075
 kkalani@just.edu.jo

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