The Association of Blood Boron Levels with Blood Cell Counts, Mineral Levels and Postnatal Nutritional Status in Premature Newborn Infants

Prematüre Yenidoğanlarda Kan Bor Düzeylerinin Kan Sayımı, Mineral Düzeyi ve Postnatal Beslenme Durumu ile İlişkisi

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ABSTRACT Objective: To investigate the relationship between whole blood boron levels of premature newborns and complete blood count, plasma calcium and phosphorus levels, postnatal nutritional status. **Material and Methods:** This study included premature neonates who were born earlier than 35 weeks and treated in the neonatal intensive care unit. We evaluated the relationship between blood boron levels and gestational week, birth weight, sex, complete blood count, plasma calcium and phosphorus levels, nutritional status, and growth. Postnatal malnutrition at the sampling day was also recorded. Patients were divided into three groups according to feeding type as breast fed, mixed fed, or parenterally fed group 1, 2, and 3, respectively. Blood was drawn from 50 premature newborns between postnatal day 21 and 23 to evaluate the relation with feeding type and blood boron levels. Blood samples were frozen at 40°C. Total parenteral nutrition (TPN) samples were collected at analysis day. All samples were analyzed for boron by colorimetric spectrophotometry. **Results:** The mean boron levels of all patients were 1.75 ± 1.11 mg/L, the median level was 1.62 mg/L, and ranged between 0.25 to 5.74 mg/L. There was no correlation with blood boron levels and gestational week, birth weight, complete blood count, or plasma calcium and phosphorus levels. Blood boron levels were higher in TPN group than others without significant difference. TPN solutions were contaminated with boron. Blood boron levels were significantly lower in the postnatal malnutrition group (p= 0.036). **Conclusion:** Blood boron levels of premature newborns were similar to adults. All components of TPN were contaminated with boron. Blood boron levels were lower in malnutrition group. Low boron levels in malnourished patients may result from insufficient intestinal absorption or excess use of boron because of high catabolism.

Key Words: Infant, newborn; infant, premature; parenteral nutrition; malnutrition; boron

ÖZET Amaç: Prematüre yenidoğanların kan bor düzeylerinin tam kan sayımı, serum kalsiyum ve fosfor düzeylerine etkisini, beslenme şekli ve büyüme ile ilişkisini incelemektir. **Gereç ve Yöntemler:** Çalışmaya 35 haftanın altında doğan ve yenidoğan yoğun bakım ünitesinde yatan prematüre yenidoğanlar alındı. Gebelik yaşı, doğum ağırlıkları, cinsiyetleri, tam kan sayımı, serum kalsiyum ve fosfor düzeyleri, postnatal malnütrisyon, beslenme ve büyüme bilgileri kaydedildi. Hastalar beslenme şekline göre sadece anne sütü, anne sütü + mama ve sadece total parenteral nutrisyon (TPN) olmak üzere 3 gruba ayrıldı. Elli hastadan postnatal 21-23. günler arasında beslenme şekli ile kan bor düzeyleri arasındaki ilişkiyi değerlendirmek için venöz kan örnekleri alındı. Kan bor düzeyi ölçümü için örnekler 40°C saklandı. TPN sıvılarının bor miktarlarını ölçmek için alınan örnekler çalışma günü laboratuvara iletildi. Bor düzeyleri kolorimetrik spektrofotometri ile ölçüldü. **Bulgular:** Prematüre yenidoğanlarda kan bor düzeyinin 0.25 ile 5.74 mg/L arasında değiştiği, ortanca 1.62 mg/L, ortalama 1.75 ± 1.11 mg/L olduğu bulundu. Kan bor düzeyi ile gebelik yaşı, doğum ağırlığı, tam kan sayımı, serum kalsiyum ve fosforu arasında ilişki saptanmadı. TPN alanların kan bor düzeyi yüksek bulundu ancak fark anlamlı bulunmadı. TPN sıvılarının bor ile kontamine olduğu görüldü. Postnatal malnütrisyon görülen bebeklerin kan bor düzeyi yeterli kilo alımı olan bebeklere göre istatistiksel olarak düşük bulundu (p= 0.036). **Sonuç:** Prematüre yenidoğanların kan bor düzeyinin erişkinlerin kan bor düzeyine benzer olduğu, TPN sıvılarının bor ile kontamine olduğu gösterildi. Postnatal malnütrisyonu olan hastaların kan bor düzeyi anlamlı olarak düşük saptandı. Neden olarak yetersiz gastrointestinal emilim veya artmış katabolizma nedeniyle fazla bor kullanımı olabileceği düşünüldü.

Anahtar Kelimeler: Yenidoğan; prematürite; parenteral nütrisyon; malnütrisyon; bor

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Boron (B) is an essential bioactive element for all vascular plants and animals but it is controversial in humans.[1] In 1981, Nielsen suggested that B might be an essential nutrient for chicks, and that B deficiency causes depressed growth.[2] B plays a role in the reproduction of flies, fish, frogs, and mice. Boron is essential for reproduction, embryogenesis, organogenesis, and development in animals.[3,4] Armstrong et al have suggested that B deficiency affects the immune response and growth in gilts.[5] B also plays a regulatory role in the metabolism of several micronutrients, such as calcium, phosphorus, and magnesium.[6]

B level in humans' affects blood cell counts and hemoglobin concentrations.[7] Experiments measuring brain and psychological function indicated that inadequate dietary B led to lower performance in cognitive and motor tests.[8] The effects of B deficiency or toxicity on newborn and premature infants are unknown. We know that B affects growth, hematological parameters, and bone metabolism; therefore boron may affect premature infants. The present study was designed to investigate the association of blood boron levels (BBL) of premature newborns with complete blood count (CBC), plasma calcium (Ca) and phosphorus (P) levels, and postnatal nutritional status. We also investigated the amount of boron in TPN solutions to evaluate the contamination.

MATERIAL AND METHODS

This study included premature neonates who were born earlier than 35 weeks and treated in the NICU of Başkent University Hospital between January and August 2005. Patients with major congenital anomaly, congenital heart disease, intracranial hemorrhage, and babies of diabetic or hypertensive mothers were excluded. Informed consent form was signed by parents and ethics committee approval was taken from Medical School of Başkent University. Gestational age, birth weight, sex, feeding and total parenteral nutrition properties, the amounts of red blood cell transfusions, weight on sampling day, CBC parameters, plasma Ca and P levels were noted.

Blood was drawn from 50 premature newborns between postnatal day 21 and 23 to check plasma electrolyte levels and CBC parameters, and 2 cc blood was also drawn into a polypropylene syringe analyze for B at same time. Postnatal 21-23 days were chosen as sampling day to evaluate the effect of feeding strategies for blood boron levels. Blood samples were frozen at -40^{O}C until analyzed for B. Eleven components used in the formulation of TPN solutions were selected for testing B concentration at analysis day. The colorimetric spectrophotometry method was chosen for determining blood boron levels; becauese of reaching easily and its cost effectivity. All blood and TPN samples were put into porcelain glasses and were ashed in a muffle furnace at 500^{O}C, and ashed samples were dissolved in dilute acid (HNO_3) before dying with azomethin-H at pH 5.1. Boric acid/azomethin-H colored complexes were analyzed for B at 420 nm wavelength absorbance by a colorimetric spectrophotometry. Because dying and temperature can affect the decomposition of B, all samples were analyzed twice.

Patients were divided into three groups according to feeding type during the last week before sampling. Infants that were breast fed, mixed fed, or parenterally fed were evaluated as group 1, 2, and 3, respectively. Patients at sampling day smaller than the 10th percentile for gestational age were evaluated as the postnatal malnutrition group.[9] The correlation between BBL and gestational week, birth weight, sex, CBC, amounts of erythrocyte transfusion, Ca and P levels, nutritional status were evaluated with Pearson correlation analyse test. The difference of BBL of groups were analyzed with Independent-samples T-test or One-way ANOVA tests.

RESULTS

Among fifty cases, BBL of 46 infants (28 girls and 18 boys) was measurable. Demographical properties of patients are listed in Table 1. The mean boron levels of all patients was 1.75 ± 1.11 mg/L, the median level was 1.62 mg/L, and ranged between 0.25 to 5.74 mg/L. Blood boron level in boys were hig-

TABLE 1: Demographical properties of patients.

Boys/Girls (n)	18/28
Gestational weeks (wk)	29.8 ± 2.0 (26-34 wk)
Birth weigth (g)	1290 ± 317 (680-1950)
Sampling day weigth (g)	1515 ± 351 (780-2350)
Hemoglobin (g/dL)	10.1 ± 1.7 (7.03-13.5)
Hematocrit (%)	28.5 ± 4.9 (18.8-37.8)
WBC count (K/mL)	12.7 ± 3.9 (5420-22600)
Platelet count (K/mL)	376 ± 181 (31-683)
Calcium (mg/dL)	9.54 ± 0.74 (7.2-11.6)
Phosphorus (mg/dL)	4.50 ± 1.74 (2.2-8.1)
Blood boron levels (mg/L)	1.75 ± 1.11 (0.25-5.74)

her than in girls, but not significantly (BBL of boys: 1.83 ± 0.96 mg/L, girls: 1.69 ± 1.19 mg/L; p> 0.05). There was no correlation with BBL and gestational week, birth weight, CBC and formulation; blood hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution weight (RDW), red blood cell count (RBC), platelet count, mean platelet volume (MPV), white blood cell count (WBC) and absolute neutrophil, lymphocyte, monocyte, eosinophil, basophil cell count, amounts of erythrocyte transfusion, plasma calcium and phosphorus levels. There were no differences in Hb concentration, Htc, MCV, MCH, MCHC, RDW, MPV, RBC, platelet count, WBC count and absolute neutrophil, lymphocyte, monocyte, eosinophil, basophil cell counts, Ca and P levels between feeding groups. BBL was higher in parenterally fed infants (group 3) but not significantly (Table 2). Blood boron levels were significantly lower in the postnatal malnutrition group than no malnutriton group (n= 14; 1.23 ± 0.65 mg/L, n= 32; 1.98 ± 1.20 mg/L respectively, p= 0.036 (Figure 1). All components of TPN were contaminated with B (Table 3). The boron content of parenteral nutrition solutions was sufficient to meet the daily demands.

TABLE 2: Results of feeding groups.

Feeding groups	Breast fed	mixed	TPN	mean
Number (n)	22	8	16	46
Boron (mg/L)	1.69 ± 0.86 (0.58-3.64)	1.52 ± 0.92 (0.36-3.64)	2.03 ± 1.48 (0.25-5.74)	1.75 ± 1.11 (0.25-5.74)
Hb (g/dL)	10.4 ± 1.4 (8.93-12.1)	10.1 ± 1.6 (7.03-12.5)	10.4 ± 2.1 (7.90-13.5)	10.1 ± 1.7 (7.03-13.5)
Htc (%)	29.5 ± 4.1 (25.1-38.3)	28.2 ± 4.9 (18.8-38.8)	29.5 ± 6.0 (22.5-37.4)	28.5 ± 4.9 (18.8-37.8)
MCV (fL)	93.8 ± 5.6 (88.7-101)	92.3 ± 6.8 (78.3-104)	92.7 ± 4.9 (84.5-97.6)	92.5 ± 6.4 (78.3-104)
MCH (pg)	33.3 ± 2.9 (30.1-37)	33.1 ± 2.6 (27.9-37.6)	32.8 ± 2.0 (29.7-34.6)	33.1 ± 2.5 (27.9-37.6)
MCHC (%)	35.5 ± 1.2 (33.9-36.8)	35.9 ± 1.0 (34.0-38.3)	35.4 ± 0.4 (34.8-36.0)	35.8 ± 1.0 (33.9-38.3)
RDW (%)	17.5 ± 1.3 (15.9-19.0)	17.1 ± 1.2 (15.0-20.2)	17.6 ± 0.8 (16.3-18.4)	17.2 ± 1.2 (15.0-20.2)
RBC (M/mL)	3.14 ± 0.45 (2.63-3.69)	3.10 ± 0.60 (2.18-4.10)	3.17 ± 0.58 (2.41-3.83)	3.11 ± 0.58 (2.18-4.10)
WBC (K/mL)	12.3 ± 2.8 (9.68-16.10)	12.9 ± 4.3 (5.42-22.6)	12.4 ± 1.3 (10.9-13.7)	12.7 ± 3.9 (5420-22600)
PLT (K/mL)	376 ± 139 (197-515)	368 ± 193 (31.3-683)	430 ± 139 (252-581)	376 ± 181 (31-683)
Calcium(mg/dL)	9.24 ± 0.54 (7.1 ± 11.2)	9.45 ± 0.37 (7.3 ± 11.2)	9.63 ± 0.77 (7.8 ± 11.5)	9.54 ± 0.74 (7.2-11.6)
Phosphorus (mg/dL)	4.66 ± 1.86 (2.6-8.1)	4.27 ± 1.67 (2.2-7.9)	4.42 ± 1.52 (2.4-8.0)	4.50 ± 1.74 (2.2-8.1)

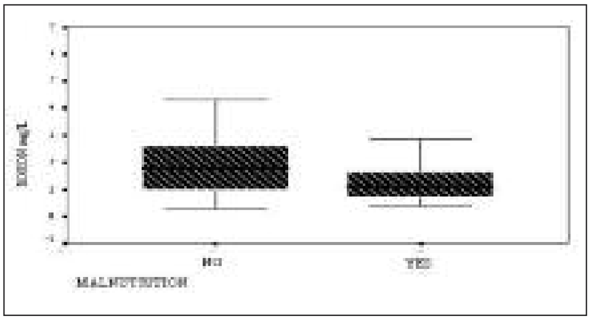

FIGURE 1: Blood boron levels and postnatal malnutrition.
BBL of the malnutrition group was significantly lower than no malnutrition group (p= 0.036).

TABLE 3: Boron contamination of TPN solutions.

Solution type	Boron amount (mg/L)
Trophamin® (6% amino acid solution)	2.274
ClinOleic® (20% lipit solution)	2.724
Dextrose (10%)	1.262
NaCl (3%)	0.812
KCl	2.387
K-phosphate	3.174
Ca-gluconate	5.760
MgSO4	3.061
NaHCO3	2.720
Tracutil® (trace elements solution)	3.170
Soluvit® (water soluable vitamins solution)	2.836

All components of total parenteral nutrition were contaminated with B.

DISCUSSION

Boron levels can depend on measurement technique. BBL were measured in adults as 1.71 ± 1.1 mg/L by colorimetric spectrophotometry.[10] The plasma B concentration in neonates ranged from 0.21 to 0.62 mg/L determined by atomic absorption spectrophotometry.[11] There is no literature data about BBL in premature infants. This study showed that the BBL of premature infants ranged from 0.25 to 5.74 mg/L, the median value was 1.62 mg/L, and the mean value was 1.75 ± 1.1 mg/L by colorimetric spectrophotometry, which was similar to adult levels determined by the same method. The mean BBL of boys was higher than girls, but not significantly, as in the literature.[12]

In rats, B supplementation reduces the white blood cell count and increases circulating concentrations of natural T cells.[13] Boron supplementation increases blood hemoglobin concentration, MCHC, and MCH, and decreases hematocrit, RBC count and platelet count in humans. Dietary B did not affect MCV, MPV, and WBC counts. These findings suggested that B affects membrane function and thus indirectly affects erythropoiesis or hematopoiesis.[7] Nielsen and Penland reported that peripheral B supplementation increases WBC count and the percentage of neutrophils, and decreases lymphocyte count in peripheral formulation.[14] In our study B levels did not correlate with CBC, absolute cell counts in peripheral formulation, or the amount of erythrocyte transfusions. All studies on the relationship between B and hematological parameters have investigated the effects of B supplementation, but no data exists about the effects of BBL on hematological parameters.

Boron also seems to affect the metabolism of Ca and P in animals and humans. Dietary B supplementation increased apparent absorption and the balance of Ca and P in vitamin D-deficient rats, increased plasma Ca in cholecalciferol-deficient chicks, increased plasma Ca, P, alkaline phosphatase in broiler chickens, decreased urinary Ca and P excretion in women consuming a low-magnesium diet, increased ionized Ca and vitamin D and decreased serum calcitonin and osteocalcin in older humans.[6,15-18] These studies suggested that if the diet is also low in other minerals or vitamin D, the level of B in diet affects mineral balance. B may affect premature infants because of their susceptibility to osteopenia and incomplete bone mineralization. There was no data evaluating BBL and mineral balance. No correlation was detected with plasma Ca, P, levels and BBL. Ca and vitamin D supplementation of all premature infants included in this study may explain this lack of correlation.

Various trace elements, including iron, zinc, copper, selenium, manganese and iodine are commonly added to formulas and TPN solutions. Ultratrace elements (boron, aluminum, molybdenum, nickel and vanadium) are not added. Nearly all of the components used for TPN solutions are contaminated with ultra trace elements.[19,20] Potential sources of trace element contamination include packaging, manufacturing methods, and inherent impurities in the chemical constituents used to produce the component solutions. Berner et al reported that solutions of Ca-gluconate, potassium phosphate, and Mg sulfate consistently contained the highest boron concentrations, but since inadvertent intake of B from TPN solutions is <10% of the amount absorbed daily by normal subjects, B deficiency does exist in long-term TPN patients.[19] Contrary to this report, Pluhator-Murton et al reported that boron contaminates all of TPN solutions, especially amino acids, potassium chloride, Ca-gluconate, and sodium chloride solutions were substantial.[20] In our study, eleven components used in the formulation of TPN solutions were selected for testing B concentration. Samples were analyzed for B using colorimetric spectrophotometry. Boron was present in every component analyzed. Ca-gluconate, potassium phosphate, Mg sulfate, and trace element (Tracutil®) solutions contained the greatest amount. However, the total amount of these solutions were lower than the total amount in TPN, indicating that the main sources of boron in TPN were dextrose and amino acid solutions. Boron contamination in TPN ranged from 0.21 mg/day to 0.56 mg/day, which approximates the recommended daily boron intake for 6- to 11-month-old infants. Mean blood boron levels were slightly, but not significantly, higher in the TPN group than the breast-fed or mixed fed groups. This excessive B may be due to the administration of boron intravenously. Trace element loading and toxicity (especially aluminum) have been reported in infants and adults who have received trace element-contaminated TPN solutions.[21] Fortunately, there are no cases of boron toxicity via TPN solutions in the literature. Although safe in adults, excessive B and/or other trace elements in TPN solutions may be toxic in premature infants because of kidney immaturity.

Recent studies showed that B supplementation to a low-B diet increased performance of chicks fed both of adequate and inadequate vitamin D-containing diets, measures of bone in barrows, and impaired the survivability of zebra fish embryos and growth of trout embryos.[18,22,23] There are no studies on the effects of B deficiency and/or B supplementation in human fetal or infant growth. Here we showed low boron levels in 14 premature newborns with postnatal malnutrition. Low boron levels in malnourished patients may result from insufficient intestinal absorption or excess use of boron because of high catabolism. Nine of these 14 premature infants were also small for their gestational age, putatively due to maternal boron deficiency or intrauterine malnutrition, which could lead to low BBL in these infants. A cohort study should evaluate the effects of both dietary B and cord blood boron levels on growth in infants of pregnant women.

The study method was the most important limitation of this study because blood boron levels were measured cross sectionally. A cohort study may ensure more valuable information about boron and growth. Also, the measurement of human milk boron levels of breast-fed premature infants could provide more quantitative values.

CONCLUSION

Blood boron levels of premature newborn infants were similar to adults. There was no correlation with blood boron level and gestational age, birth weight, complete blood count and formulation, and plasma calcium and phosphorus levels. Blood boron levels in parenterally fed infants were slightly higher than breast-fed or mixed-fed infants, probably due to TPN contamination with boron. BBL were lower in the malnutrition group. Evaluation of boron levels of cord blood and BBL in pregnant women with a cohort study will ensure more valuable information about boron and growth.

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