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Clinical studies

Serum selenium levels of the very low birth weight premature newborn infants with bronchopulmonary dysplasia

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ABSTRACT

Background: The selenium (Se) is an essential trace element that has a critical role in synthesis and activity of a number of selenoproteins with protective properties against free radical damage. This study was conducted to detect the serum Se concentration in very low birth weight (VLBW) preterm infants and its association with bronchopulmonary dysplasia (BPD).
Materials and methods: Cord blood Se concentration was determined in 54 neonates with gestation age 30 week or less. Another sample was obtained from these infants at day 28 of birth and serum Se levels were measured by atomic absorption spectrophotometer. All neonates were followed for oxygen dependency at 28 day after birth and 36 week postmenstrual age.
Results: The mean cord blood Se concentration in studied neonates was $64.78 \pm 20.73\ \mu g\ L^{-1}$. Serum Se concentration was $60.33 \pm 26.62\ \mu g\ L^{-1}$ at age 28-day. No significant correlation was observed for serum Se concentration at birth and at one month after birth ($r = -0.04$, $p = 0.72$). BPD was diagnosed in 25 neonates (46%). The mean serum Se concentration at one month was $57.16 \pm 29.68\ \mu g\ L^{-1}$ in patients with BPD (25 cases) and $63.27 \pm 23.6\ \mu g\ L^{-1}$ in 29 patients without BPD ($p = 0.40$).
Conclusion: In our study, serum Se concentration at 28 day of birth was lower than cord blood levels in preterm neonates, but we have not found significant difference among patients who had BPD or not with respect to serum Se concentrations at this age.

Introduction

Trace elements are necessary for metabolism, growth and neurologic or immunologic functions [1]. The selenium (Se) is an essential trace element, of importance to human biology and health, that has a critical role in synthesis and activity of a subset of enzymes called selenoenzymes that the most important of them is glutathione peroxidase. This enzyme protects cells and tissues against free radical damage by antioxidant properties [2,3]. Several diseases are caused by selenium deficiency including Kashan disease (a fatal cardiomyopathy), Kashin–Beck disease (a chondrodystrophy), increased erythrocyte fragility in preterm infants, muscle pain, weakness, and myopathy; macrocytosis, alopecia, pseudoalbinism, growth retardation and progressive encephalopathy [4–6] numerous reports implicate selenium deficiency in several reproductive and obstetric complications [7]. The maternal transplacental transfer of Se to fetus is limited [8,9]. Se is stored in fetal liver between 20th and 40th week [10]. The plasma Se concentration in preterm infants is lower than adults.

Several techniques have been developed for Se determination in serum, plasma and whole blood. These methods include atomic spectroscopic techniques such as electrothermal atomic absorption (ET-AAS), atomic absorption with hydride generation (AAS-HG), atomic fluorescence with hydride generation (AFS-HG), molecular fluorescence spectrometry (FS) neutron activation analysis (NAA), inductively coupled plasma mass spectrometry (ICP-MS) and isotope dilution mass spectrometry (ID-MS) [11–16]. Owing to the low limit of detection, selectivity, sensitivity and minimum sample quantity, ET-AAS is more accessible to chemical and biochemical laboratories. ET-AAS is employed in this work and the validation process was conducted to ensure the validity of the generated data.

The preterm infants with very low birth weights (VLBW) have given metabolic characteristics that predispose them to free radical damage including bronchopulmonary dysplasia (BPD) and

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Table 1
Temperature program for Se analysis in serum samples by ET-AAS.

Stage	Temperature (°C)	Time (s)		Argon gas flow ($L\,min^{-1}$)
		Ramp	Hold	
Drying (1)	110	10	10	1.5
Drying (2)	140	10	10	1.5
Pyrolysis	800	10	20	1.5
Atomization	2200	0	3	0.0
Cleaning	2800	1	1	1.5

retinopathy of prematurity (ROP) [17,18]. Advanced care of preterm infants has been resulted to increased survival rate of infants with lower gestation age and birth weight.

BPD was first reported in 1967 by Northway et al. [19]. They described the disorder in premature infants with respiratory distress syndrome (RDS) who underwent prolonged mechanical ventilation with high pressure and FiO_2. A new BPD has been described recently that is milder than the more advanced stages of the traditional disease. BPD is one of the most challenging chronic diseases of prematurity. There is considerable variability in the reported incidence of BPD in part due to use of different diagnostic criteria [20]. There are several proposed etiologies for BPD. The incidence of BPD increases as birth weight decreases [21]. Prolonged exposure to high FiO_2 and free oxygen radicals can cause tissue damage of BPD [21,22]. Lung damage could be increased in the presence of inflammatory process [21,23]. Nutrient deficiency also plays an important role in the development of BPD by reducing antioxidant function, predisposing to infection and impaired lung repair [22,23]. Because of a large number reported diseases and complications in premature newborn infants, appropriate attention is needed in care and nutrition of these vulnerable infants

Supplementation with Se is suggested after 2 weeks of age because preterm infants can become Se deficient after 2 weeks of exclusive parenteral nutrition. The recommendation for Se supplementation of total parenteral nutrition for premature infants is not routine in Iran. There is limited biochemical reference data on Se status in VLBW preterm infants. The aim of this study was to detect the serum Se concentration in VLBW preterm infants and its association with BPD as a chronic lung disease.

Experimental

Apparatus and instruments

Atomic absorption spectrophotometer Model CTA 3000 was used for the determination of Se, equipped with a transversely heated graphite atomizer (THGA) and a circulating cooling unit. A Se hollow cathode lamp (operated at 5 mA) was applied as the radiation source at the wavelength of 196.1 nm with 0.5 nm spectral band pass. Deuterium lamp background correction was employed to correct for the non-specific absorbance. Argon 99.999% (Roham gas Co., Tehran, Iran), with a $1.5\,L\,min^{-1}$ flow rate, was used as protective and purge gas. Aliquots of 25 μL for all samples and calibration solutions were injected directly into the graphite tube by the micro-sampler. The details of graphite furnace temperature program used for the determination of Se are listed in Table 1. Atomic signal measurements were performed in the peak area mode. This mode was preferred over peak height, since the latter gives low reproducible results.

All chemicals used were of analytical-reagent grade and all solutions were prepared with ultra-pure deionized water (Shahid Ghazi Co., Tabriz, Iran). The working solutions of Se(IV) were prepared daily by stepwise diluting the stock standard solution of $1000\,mg\,L^{-1}$ (Merck, Germany), and shaking them just before use. Nitric acid, hydrogen peroxide and all salts used, were purchased from Merck. One thousand $mg\,L^{-1}$ of Ni, Cu and Mg (nitrate salts, Merck) were tested as chemical modifiers.

Preparation of serum samples

Serum samples were collected in the neonatal intensive care unit of Al-Zahra Hospital, a university level III neonatal center. Umbilical cord bloods were collected immediately after cutting off the cord. Another blood sampling was performed 28 days after birth for serum Se measurements. The samples were centrifuged at 3000 rpm and stored at −70 °C until analysis by a biochemical staff that had no idea about the patients.

Digestion method

Se is found in the serum samples mainly as selenoproteins. In order to release it from these proteins, several wet acid digestion methods were tested and the obtained results using a mixture of nitric acid and hydrogen peroxide were found as the best digestion method for Se analysis in serum samples [24].

Measurement procedure

Aliquot of 100 μL from each serum sample was place in a 25 mL glass beaker. Then, 10 mL of concentrated HNO_3 and 6 mL of 30% (v/v) H_2O_2 solution were added to sample and the solution heated in oil batch at 140 °C to complete digestion of the sample until dryness. After cooling, the residual was dissolved by 200 μL of 15% (v/v) HNO_3. Afterward, 50 μL of $1000\,\mu g\,mL^{-1}$ nickel nitrate was added into obtained solution as a chemical modifier. Finally, 25 μL of the resultant solution was injected into the graphite furnace atomizer and submitted to the temperature program listed in Table 1. Measurements of each analytical solution and samples were carried out in triplicates.

Study population

A prospective observational longitudinal study conducted and premature newborn infants whose gestation age was 30 weeks or less were recruited for this study. Exclusion criteria were twin or triple delivery, neonates with major congenital malformations, and suspected chromosomal anomalies.

Gestational age was determined by the neonatologist based on the first trimester ultrasound examination, neonatal physical examination and Ballard scoring [25]. All studied neonates had birth weight less than 1500 g and considered as VLBW infants. They were fed either breast milk that was fortified when its volume reach 120 mL per kg per day or premature formula, started as soon as clinical stabilization. None of the patients received supplemental parenteral Se during hospitalization. Respiratory distress syndrome (RDS) was diagnosed based on classic clinical signs and confirmed radiologic findings. Patients were followed until discharge or at least one month after birth. Criteria for BPD, included those infants who required treatment with FiO_2 more than 21% oxygen for at least 28 days. The ethic committee of the university authorized the study and written informed consent was obtained from the parents.

Statistical analysis

Statistical analyses were carried out using SPSS package 15. Student's *t*-test or Mann–Whitney *U*-test and chi squared test were used for quantitative and qualitative variables respectively. Spearman rank correlation coefficient and regression analysis were used