Clinical studies

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

Ali Peirovifar a, Manizheh Mostafa Gharehbaghi b,∗, Hossein Abdulmohammad-zadeh c, Gholam Hossein Sadegi d, Abulghasem Jouyban e

a Department of Anesthesiology, Tabriz University of Medical Sciences, Tabriz, Iran
b Women’s Reproductive Health, Research Center, Department of Pediatrics, Tabriz University of Medical Sciences, Tabriz, Iran
c Department of Chemistry, Faculty of Science, Azerbaijan University of Tarbiat Moallem, P.D. Box 53714-161, Tabriz, Iran
d Tuberculosis and Lung Disease Research Center, Tabriz, Iran
e Drug Applied Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

A R T I C L E   I N F O
Article history:
Received 15 July 2012
Accepted 13 March 2013

Keywords:
Selenium
Cord blood
Preterm infants
Bronchopulmonary dysplasia

A B S T R A C T

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 ± 20.73 μg/L. Serum Se concentration was 60.33 ± 26.62 μg/L 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 ± 29.68 μg/L in patients with BPD (25 cases) and 63.27 ± 23.6 μg/L 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.

© 2013 Elsevier GmbH. All rights reserved.

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) and neutron activation analysis (NAA), inductively coupled plasma mass spectrometry (ICP-MS) and isotopic 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.

Se concentration in preterm infants is lower than adults. 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...
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 FiO2. 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 FiO2 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.

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 HNO3 and 6 mL of 30% (v/v) H2O2 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) HNO3. Afterward, 50 μL of 1000 μg 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 FiO2 more than 21% oxygen for at least 28 days. The ethical 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.

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

| Stage        | Temperature (°C) | Time (s) | Argon gas flow (L min

−1) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Drying (1)</td>
<td>110</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Drying (2)</td>
<td>140</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Pyrolysis</td>
<td>800</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Atomization</td>
<td>2200</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Cleaning</td>
<td>2800</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Criteria**

- **Temperature (°C)**: The temperature levels used in the experiment.
- **Time (s)**: The duration for which the temperature was applied.
- **Argon gas flow (L min

−1)**: The flow rate of argon gas used to assist the atomization process.
to relate selenium levels and clinical data. p values less than 0.05 were considered to be statistically significant.

**Results**

**Optimization of the ET-AAS conditions**

The ET-AAS technique is a powerful tool to determine total Se at very low levels, which can be suitable for application in several kinds of samples. In order to reduce the interferences and increase the accuracy, the use of a chemical modifier or a modifier mixture has become indispensable in ET-AAS measurements. Therefore, the use of matrix modifier becomes highly necessary due to the volatility of Se compounds. In this work, when the chemical modifier was not employed, the analytical signal was gradually decreased until 70% of the initial signal. Thus, in order to choose the maximum permissible pyrolysis temperature and the optimal mass of the modifier, three nitrate salt solutions of nickel, copper and magnesium with different concentrations were individually tested as chemical modifier for the determination of Se. According to the results, addition of 200 µg mL⁻¹ of Ni solution allowed increasing the analytical signal with considerable background reduction, without increasing the pyrolysis temperature.

The pyrolysis and atomization curves were respectively plotted to determine the effects of pyrolysis and atomization temperature in the analysis of Se in the standard solutions containing 160 µg L⁻¹ Se and 200 µg mL⁻¹ Ni as a chemical modifier. The results (details are not shown) revealed that 800 and 2200 °C are the optimized pyrolysis and atomization temperature, respectively. Using the optimized temperatures, the effects of pyrolysis and atomization time on the absorbance of Se were also investigated. Based on the obtained results, a pyrolysis time of 30 s was sufficient to produce the maximum analytical signal. However, the atomization time had little effect on the atomic signal of Se. So, 30 and 3 s were selected as pyrolysis and atomization time, respectively.

**Analytical performance**

Using optimized conditions, analytical curve was plotted in the concentration range of 5–500 µg L⁻¹ of Se. The calibration graph was linear from 5 to 400 µg L⁻¹ with a correlation coefficient of 0.995. The regression equation was $A = 0.001 C_{Se} + 0.018$, where $A$ is the absorbance and $C_{Se}$ is the Se concentration expressed in µg L⁻¹, respectively. The limit of detection, calculated as three times the standard deviation of the blank signal, was 2.0 µg L⁻¹. The relative standard deviation (RSD) resulting from the analysis of 6 replicates of Se solution containing 100 µg L⁻¹ Se(IV) was 2.9%.

**Analysis of real samples**

The presented method was employed to determine the trace amounts of Se in the collected serum samples. In order to verify the accuracy of the established procedure, recovery experiments were carried out by spiking the samples with different amounts of Se(IV) before any pretreatment. Based on the obtained results, the recovery values were between 93.4 and 97.6% (Table 2), which confirm the accuracy of the developed method.

**Clinical findings**

The study group consisted of 54 preterm newborn infants with mean gestation age of 29.14 ± 1.21 (range: 25–30) week and birth weight 1172 ± 245 (range: 710–1500) g. The mean maternal age was 27.95 ± 6.02 (range: 18–41) year.

The mean cord blood Se concentration in studied neonates was 64.78 ± 20.73 µg L⁻¹ (range: 15.2–129). Serum Se concentration at 1 month age was 60.33 ± 26.62 µg L⁻¹ (range: 8.9–119.9). There was no significant correlation between either Se levels at birth ($r = 0.09, p = 0.51$) or serum Se concentration one month after birth ($r = 0.11 p = 0.42$) and gestation age of the infants. No significant correlation was observed for serum Se concentration at birth and at one month after birth ($r = −0.04, p = 0.72$). Twenty-two infants (47%) were girl. BPD was diagnosed in twenty-five (46%) preterm infants that 9 of them were girl ($p = 0.44$).

Surfactant replacement therapy was done in 27 neonates (50%) and mechanical ventilation in 12 neonates (22%). The mean cord blood Se concentration in infants with RDS was 63.27 ± 29.54 µg L⁻¹ and in preterm infants without RDS was 66.30 ± 24.06 µg L⁻¹ ($p = 0.68$). The mean serum Se levels one month after birth was 57.85 ± 26.64 µg L⁻¹ in patients with RDS and 62.81 ± 26.88 µg L⁻¹ in patients without RDS ($p = 0.49$). The mean serum Se concentration at one month was 57.16 ± 29.68 µg L⁻¹ in patients with BPD (25 cases) and 63.27 ± 23.66 µg L⁻¹ in 29 patients without BPD ($p = 0.40$).

Selenium concentration and other characteristics of patients with BPD are shown in Table 3 and compared with infants without BPD.

**Discussion**

The average cord blood Se concentration has been reported 35–107 µg L⁻¹ [26–31] depending on the selenium content of soil in

<table>
<thead>
<tr>
<th>Variable</th>
<th>BPD group (n = 25)</th>
<th>Infants without BPD (n = 29)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestation age (week)</td>
<td>27.69 ± 1.78</td>
<td>30.5 ± 1.66</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight (gram)</td>
<td>1062 ± 237</td>
<td>1273 ± 209</td>
<td>0.001</td>
</tr>
<tr>
<td>Maternal age (year)</td>
<td>27.95 ± 6.02</td>
<td>27.85 ± 6.28</td>
<td>0.95</td>
</tr>
<tr>
<td>Duration of CPAP (day)</td>
<td>5.84 ± 1.00</td>
<td>2.46 ± 0.39</td>
<td>0.004</td>
</tr>
<tr>
<td>Duration of MV (day)</td>
<td>3 ± 1.2</td>
<td>0.28 ± 0.15</td>
<td>0.02</td>
</tr>
<tr>
<td>First day fluid intake, ml kg⁻¹ day⁻¹</td>
<td>98.8 ± 12.85</td>
<td>88.1 ± 11.7</td>
<td>0.006</td>
</tr>
<tr>
<td>7th day fluid intake, ml kg⁻¹ day⁻¹</td>
<td>187.8 ± 36.05</td>
<td>170.36 ± 30.39</td>
<td>0.06</td>
</tr>
<tr>
<td>Cord blood Se, µg L⁻¹</td>
<td>69.82 ± 28.47</td>
<td>60.11 ± 24.59</td>
<td>0.18</td>
</tr>
<tr>
<td>Serum Se at 1 month, µg L⁻¹</td>
<td>57.16 ± 29.68</td>
<td>63.27 ± 23.66</td>
<td>0.40</td>
</tr>
</tbody>
</table>

MV: mechanical ventilation; CPAP: continuous positive airway pressure.
different regions. Wells et al. reported mean umbilical cord Se concentration 70.10 μg L⁻¹ with 95% confidence interval 68.69–70.52 among urban newborns. Asian neonates had 6.58 μg L⁻¹ lower Se concentration in umbilical cord than Caucasian infants [28].

Makhoul et al. examined Se in the umbilical cord blood of newborns with gestation age of 24–42 weeks and found a significant association between gestation age and serum Se concentration after 36 weeks [32].

Mentro examined eighteen preterm infants of 30 week gestation age or less at postnatal age 1 and 4 week in a Se adequate area and showed decreased plasma and erythrocyte Se concentrations from week 1 to week 4 [17]. It was 0.97 ± 0.21 μmol L⁻¹ (range: 0.62–1.58) at one week and 0.72 ± 0.27 μmol L⁻¹ (range: 0.32–1.33) at week 4. Marriott [27] determined trace elements in 68 preterm infants with gestation age of 31.4 ± 2.9 week. At week 40 corrected gestation age (CGA), plasma Se concentration was 0.49 ± 0.15 μmol L⁻¹ and girls had higher erythrocyte and whole blood Se concentrations at 1 and 6 month after birth in their study. They explained this finding by more physiologic needs of boys for Se supplementation. They suggested that because of limited transplacental transfer of Se due to preterm labor, the preterm infants have decreased liver stores of Se that predispose them to Se deficiency in neonatal period. On the other hand prematurity is associated with more oxidative stress, which can cause oxidative damage of organs like lungs or retina. It is likely that Se stores are used for production of selenoproteins. We couldn’t find any association between serum Se concentration and gestation age with no gender difference in Se levels that may be due to this fact that our studied neonates had gestation age less than 30 week. Although the selenium concentration at age 28 days of birth was lower than cord blood, it was not lower than the reported low Se serum concentration in preterm infants (it ranges from less than 40 to less than 10 μg L⁻¹) [33]. The absence of a low serum Se concentration in our study population may point to sufficient Se content in breast milk and early nutritional support of infants with breast milk that met Se requirements of neonates.

The selenium content in food depends on selenium content of the soil in which plants are grown and animals are fed. Selenium in the soil of high plains is more and it tends to concentrated in the soils of drier and more alkaline regions of the world. Our studied patients were from high altitude region with cold and dry weather. Maternal serum and breast milk Se levels were not measured in our study.

The role of Se status for BPD has been examined in a few studies and remains unclear [34–36]. Darlow et al. in 1995 studied preterm infants less than 1500 g and 32 weeks, gestation age and demonstrated the association between Se deficiency and increased respiratory morbidity [34].

Darlow and Austin [36] has conducted a meta analysis and included 297 newborn infants who received Se supplementation and 290 controls. They assessed infants with respect to the need for oxygen at day-28 and 36 week gestation corrected age, retinopathy of prematurity, episodes of sepsis, and mortality during hospitalization. They reported that Se supplementation was associated with reduction in one or more episodes of sepsis, but no significant improvement was found for survival or other studied diseases including ROP, BPD and apnea [36].

As it is showed in Table 3, preterm infants with lower gestation age and lower birth weight with more respiratory support requirements were more oxygen dependent on day 28 of life.

The serum Se concentration is readily available but has limited significance in assessing body stores. With considering life span of red blood cells (RBC), low body stores are indicated by RBC content after 2–3 months. The limitation of our study was the lack of measurement of glutathione peroxidase activity or erythrocyte Se concentration and their relationships with serum Se concentration and BPD.

Conclusion

Our study showed oxygen dependent preterm infants had lower birth weight and gestation age with more respiratory support requirements. Serum Se concentration in preterm infants with gestation age less than 30 weeks was lower at day 28 than cord blood. In patients who were oxygen dependent at day 28, Se level was lower than infants without oxygen dependency but difference was not statistically significant. It is recommended future studies with larger number of patients to determine Se status at 36 week gestation corrected age, Se status in mothers and selenium content in breast milk; and to clarify Se role in BPD.

Conflict of interest statement

None.

Acknowledgments

The authors would like to thank research vice chancellor of Tabriz University of Medical Sciences and Women’s Reproductive Health Research Center (WRHRC) for providing the funding for the study. We thank Dr. Ghanbari F., MD, Mr. Neginfar E., Mrs. Atayan R. and Mrs. Esmaeili F. for their assistance in samples collection and preparation. We also gratefully acknowledge the work done by the staff at the neonatal intensive care unit and neonatal unit, Al-Zahra hospital, Tabriz, Iran.

References


