*Original Article*

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# The Investigation of Effects of Blood Exchange Transfusion on Selenium in Newborn Infants by Instrumental Neutron Activation Analysis Method

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## Abstract

***Objective:*** The evidence for the effects of blood exchange transfusion on selenium (Se) in newborn infants is unknown. This study was conducted to determine the possible effects of blood exchange transfusion on Se by comparing the Se blood concentrations before and after exchange transfusion in jaundiced neonates.

***Methods:*** A total of 30 jaundiced term neonates who underwent blood exchange transfusion (EXT) for first time because of idiopathic unconjugated hyperbilirubinemia, were recruited. The Se level of 30 blood bank donors' samples used for EXT were measured and 30 pairs of uncontaminated umbilical cord blood samples were investigated for Se before and after exchange transfusion. The samples were analyzed by instrumental neutron activation analysis method. Serum bilirubin concentrations were measured by venous blood samples before EXT.

***Findings:*** The average of Se concentration before EXT was higher than that after EXT (629.78±283.82 SD ppb versus 454.83±213.75 SD ppb) ($P$<0.05). There was significant correlation between the blood concentration of Se before and after EXT and also between the blood level of Se before EXT and total serum bilirubin level ($P$<0.05). There was no significant correlation between the blood concentration of Se before EXT and babies' gender and weight ($P$>0.05). The average Se level in samples obtained from transfused blood products was 507.90±223.56 SD ppb.

***Conclusion:*** Blood exchange transfusion caused a 28% decrease of the blood Se level because the blood donors had lower blood Se levels than the newborns. Furthermore, there was a significant correlation between the blood level of Se before EXT and the total serum bilirubin level.

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***Key Words:*** Trace elements; Exchange transfusion; Newborn; Neutron activation analysis; Selenium

## Introduction

Fifteen trace elements are claimed to be essential for human organism. Inadequate intake of these elements causes deficiency diseases. However, even essential trace elements may become toxic at high doses[1]. Also low fetal stores, immaturity of digestive system, low content in breast milk, rapid growth and disease affect the serum concentrations[2]. The role of micronutrients deficiencies in increasing or exacerbating the major clinical problems facing neonatal health professionals in developing countries is unknown. Micronutrients or minerals levels might affect the

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role of bilirubin binding proteins or process of bilirubin excretion[3]. The antioxidant capacity of jaundiced infants were related to the level of bilirubin as a decrease in antioxidant capacity after exchange transfusion has been reported[4].

Se (Se) is an essential trace element of tremendous importance for human health. Its role in animal nutrition was first recognized in 1957 by Schwartz and Foltz who demonstrated that Se deficiency led to necrotic degeneration of liver, pancreas, heart and kidney in mammals. In humans, it was not until 1973, however, that selenium's biochemical function as an integral part of the antioxidant enzyme glutathione peroxidase (GSH-Px) was discovered[5]. Little is known about in utero transport of Se across the placenta or even distribution of Se within the fetal body. Backward extrapolation to expected accumulation in the third trimester would be 1 µg/kg/day[5]. Babies are born with lower Se concentration in their blood than their mothers' and lower still in preterm infants[6]. Infants born prematurely have lower hepatic Se stores than term infants at birth[5].

Se deficiency is characterized by peripheral myopathy with muscle weakness and muscle pain, cardiomyopathy, arrhythmias and congestive heart failure[7]. An endemic cardiomyopathy occurring mainly in infants and children in China, known as Keshan disease has been reported[5,8]. There are reports of white finger nails and increases in serum ALT (alanine aminotransferase) and AST (aspartate amino-transferase) and creatinine kinase attributed to Se deficiency during long term parenteral nutrition[5,8], and Se may be related to disturbances in thyroid hormones metabolism[9]. **Se** is an essential constituent of various enzymes including glutathione peroxidase, which protects against oxidative injury. In the neonate, neuronal injury in hypoxic ischemic encephalopathy and several other diseases is believed to be caused by oxygen free radical injury[10]; also low Se levels were associated with increased risk of fetal death, neonatal death and HIV transmission through the intrapartum route[11].

Removal of blood from the affected infant and replacing with fresh blood from blood bank (exchange transfusion) is used as a treatment for severe unconjugated hyperbilirubinemia in new-born infants. Traditionally twice the blood volume of baby is removed and replaced with fresh blood. Exchange transfusion can be associated with serious adverse events and death.

Exchange transfusion rapidly produced variable changes in the concentrations of prooxidant and antioxidant substances in plasma and may thus influence Se level in the newborn. The evidence for the effects of blood exchange transfusion on Se in newborn infants is unknown. Instrumental Neutron Activation Analysis Method (INAA) was the standard, sensitive, relatively cheap, non-destructive, powerful and most reliable analytical method with minimum detection limits in the sub-PPM range[12-14], thus INAA is performed at specialized centers, often for research. The present study aimed to elucidate the Se blood concentrations in jaundiced newborns that underwent EXT and possible effects of EXT on this selected trace element by INAA method.

## *Subjects and Methods*

The study carried out in the national laboratory of Payame Noor University and Atomic Energy Organization of Iran (AEOI) and the Tehran University of Medical Sciences, Children's Medical Center which provides levels 2 and 3 of neonatal care. This is a prospective, case series, descriptive and analytical study in Iranian newborns from October 2007 through October 2008. A total of 30 term (37-40 weeks gestation) jaundiced newborn infants who needed blood exchange transfusion admitted to Children's Medical Center, were selected. The local institutional ethics committee approved it and informed patient's parent consent was obtained. The babies who were born with history of birth trauma, asphyxia, hemolytic jaundice, major congenital malformations, and sepsis were excluded. The newborns fed with formula or milk fortifier, enteral supplement, parenteral nutrition with trace elements, as well as contaminated blood sample also were excluded from the study. The mothers with history of smoking, drug abuse, vegetarians and those with

medical condition needing treatment were excluded from the study. All infant demographic findings were recorded. Serum bilirubin concentrations were measured by venous blood samples of all patients before EXT.

Thirty pairs of umbilical cord blood samples (~5ml) from 30 newborns were obtained, one sample before EXT, and second sample 2-5 minutes after EXT were collected needleless via the umbilical vein catheter. 30 samples of transfused blood product were obtained and 5 ml blood samples from each donor were collected. All samples were collected in uncontaminated polyethylene tubes. Within few minutes the blood samples were transferred to special glass bottles. All glassware and bottles used for collection and analysis were pre cleaned with analytical grade nitric acid solution and rinsed with ion free water at PNU (Payame Noor University) national laboratory. All samples were stored at -20°C until freezing. After drying the blood samples (in Pyrex dishes with 5 cm diameter in an oven at 60°C for 7 h), samples were powdered by a porcelain mortar, 30 mg from each sample sealed in cylindrical polyethylene capsules, and distributed in irradiation tubes. Samples were irradiated in AEOI reactor laboratory by swimming pool research reactor facilities in thermal neutron flux 1-5×$10^{11}$ $cm^{-2}$ $s^{-1}$ for 59-65 minutes. Decaying of short half life trace elements took 7-10 days, and then samples were measured with an HPGe detector. They were processed in batches of 7 samples and one sample for internal quality control. The standard control reference materials were NIST-SRM-$bo_1$ (solid), NIST-SRM-$bo_2$ (solid) and NIST-SRM-OC575154 (liquid). The measurements were done in two stages, the first stage was after 10-15 days and the second stage was 22-28 days decay. The irradiated standard reference materials were measured in the same conditions and the net area of peaks were corrected according to samples and standard reference elements. The data were analyzed by Gamma-2000 and Spam software.

The independent simple t-test, one sample t-test, paired sample t-test, analysis of variance (AnoVa) test, post hoc tests (multiple comparisons, Tukey HSD), and Levine's test for equality of variances were used for data analysis. A *P*-value below 0.05 was considered significant.

## Findings

Of the 30 term neonates, 20 (66.6%) were males and 10 (33.3%) females, 14 (46.6%) mothers were primigravida. Newborn characteristics and serum bilirubin levels (Table 1) and Se status before and after blood exchange transfusion and in donors' blood (Table 2), comparison of average and standard deviation of Se levels before and after EXT with donors' blood (Table 3) of the subjects are presented. The mean postnatal age, gestational age, weight of patients, and serum bilirubin concentrations were 5.2 days, 38.5 weeks, 2950 g, and 23.5 mg/dl respectively. The mean and standard deviation of dry blood Se concentration before EXT were higher than after it (629.78±283.82 SD ppb vs 454.83±213.75 SD ppb). There was significant difference between the blood concentrations of Se before and after EXT ($P<0.05$), post EXT Se was decreased by 28% and there was a significant correlation between the blood levels of Se before EXT and total serum bilirubin levels ($P<0.05$). The mean Se concentrations before EXT in boys were 631.39±306.20 SD ppb and in girls 626.56±248.21SD ppb. The mean Se concentrations after EXT in boys were 477.93±230.57 SD ppb and in girls 408.62±177.30 SD ppb. There was no significant relation between the blood concentrations of Se before EXT and baby's sex and weight ($P>0.05$). Samples obtained from transfused blood product showed mean Se 507.90±223.56 SD ppb.

## Discussion

To our best knowledge this is the first report on this effect of EXT on Se level in newborn infants. This study showed that post EXT Se level decreased by 28%.

This study showed that adult blood bank donors had lower Se level than newborns and exchange is another factor that influences Se status in newborns. We found that the level of Se in dry blood components of healthy volunteers of blood bank donors in Tehran ranged between

**Table 1:** Newborn characteristics and serum bilirubin levels

| Patients No | Sex | Age at time of EXT (day) | Weight (gr) | Serum bilirubin before EXT (mg/dl) |
|---|---|---|---|---|
| 1 | female | 3 | 2200 | 24.0 |
| 2 | male | 5 | 2500 | 26.0 |
| 3 | male | 7 | 3000 | 21.0 |
| 4 | female | 8 | 3400 | 23.0 |
| 5 | female | 5 | 3650 | 23.0 |
| 6 | male | 6 | 2600 | 23.0 |
| 7 | male | 3 | 3900 | 23.8 |
| 8 | male | 7 | 2500 | 23.0 |
| 9 | male | 7 | 3350 | 26.5 |
| 10 | male | 4 | 2750 | 24.1 |
| 11 | male | 4 | 2500 | 26.0 |
| 12 | female | 5 | 4000 | 27.0 |
| 13 | female | 3 | 2420 | 21.6 |
| 14 | male | 7 | 2800 | 23.2 |
| 15 | male | 9 | 3300 | 27.0 |
| 16 | male | 5 | 2550 | 17.0 |
| 17 | male | 5 | 2380 | 23.3 |
| 18 | male | 4 | 3050 | 22.0 |
| 19 | female | 9 | 2000 | 16.0 |
| 20 | male | 7 | 3020 | 26.0 |
| 21 | male | 7 | 3150 | 25.0 |
| 22 | female | 3 | 3400 | 27.0 |
| 23 | female | 4 | 3300 | 21.0 |
| 24 | female | 7 | 2740 | 16.0 |
| 25 | male | 3 | 2200 | 22.0 |
| 26 | male | 5 | 2250 | 31.0 |
| 27 | male | 4 | 3600 | 25.0 |
| 28 | female | 3 | 2950 | 27.6 |
| 29 | male | 5 | 3760 | 23.0 |
| 30 | male | 4 | 3100 | 22.4 |

507.90±223.56 SD ppb. This result is close to those reported by Bakir et al, namely 650-2260 ppb (dry form)[13], but it is more than the serum levels which are reported of healthy individuals living in Tehran (100.6±12.6 ppb)[15]. The blood Se level varies widely with geographical area, country, region, sex, age, climate and season[8]. The technique appears to have significant influence on level of measured Se in serum/plasma [16]. In the present study these differences in Se status are probably due to measurement technique.

Term infants at birth have higher serum or plasma Se concentrations than their preterm counterparts and both groups have significantly lower Se levels than their mothers, and Se concentrations are generally 50-65% of those of adults[10,8]. The plasma Se concentration of the majority of healthy neonates from birth up to 3 months of age fall within the range of 50-150 ppb[5]. Our results show that in newborns Se levels before EXT was 629.78±283.82 SD ppb, it is close to the Se levels in German neonates[17], but it is more than in Indian infants, Iranian children, Italian, Iranian and Turkish newborns[5,8,18-20]. It is known that human infants are born with Se reserves but its level also depends on the Se concentration of human milk, colostrum contains more than twice the Se concentration of mature milk[5,11,16]. Although interpretation and comparison of Se concentration in neonates is difficult[5], the Se status was found to vary with postnatal age and the type of feeding[8]. In this study there was no significant correlation between blood concentrations of Se before EXT and babies' gender and weight ($P$>0.05), while Se level in newborns was higher than that of adults of

**Table 2:** Comparison of selenium (Se) status in jaundiced newborns before and after blood exchange transfusion with Se status of the donor's blood

| No | Se Before EXT (ppb) | Se Donor (ppb) | Se After EXT (ppb) | No | Se Before EXT (ppb) | Se Donor (ppb) | Se After EXT (ppb) |
|---|---|---|---|---|---|---|---|
| 1 | 802.91 | 663.21 | 387.70 | 16 | 1285.30 | 432.75 | 712.03 |
| 2 | 801.52 | 754.55 | 554.59 | 17 | 332.83 | 850.89 | 408.94 |
| 3 | 1041.70 | 343.45 | 675.23 | 18 | 126.65 | 552.19 | 237.89 |
| 4 | 638.91 | 567.88 | 668.00 | 19 | 818.74 | 457.65 | 372.25 |
| 5 | 880.72 | 502.76 | 339.67 | 20 | 449.01 | 453.02 | 337.29 |
| 6 | 633.67 | 281.90 | 868.28 | 21 | 207.19 | 475.33 | 347.84 |
| 7 | 1277.00 | 274.10 | 940.82 | 22 | 270.63 | 163.43 | 268.48 |
| 8 | 646.85 | 454.83 | 695.77 | 23 | 221.33 | 264.45 | 117.35 |
| 9 | 425.81 | 729.83 | 491.84 | 16 | 842.10 | 957.84 | 726.41 |
| 10 | 805.23 | 538.83 | 238.59 | 24 | 500.68 | 654.53 | 692.24 |
| 11 | 593.41 | 830.48 | 335.31 | 25 | 824.69 | 102.00 | 228.96 |
| 12 | 382.17 | 569.01 | 428.22 | 26 | 463.36 | 730.72 | 610.24 |
| 13 | 783.47 | 566.22 | 353.58 | 27 | 624.57 | 127.38 | 424.5 |
| 14 | 581.65 | 239.49 | 189.57 | 28 | 546.45 | 334.70 | 207.33 |
| 15 | 530.85 | 746.29 | 285.17 | 29 | 553.99 | 617.43 | 500.66 |

Tehran's blood bank donors. we do not have an explanation for this, but the possible reason for higher dry blood Se levels in babies compared to those of blood donors may be transport of Se across the placenta and with breast milk.

Low serum or plasma Se concentrations and decreased erythrocyte glutathione peroxidase (GSH-Px) activity have previously been shown to be associated with a variety of clinical problems that improved when Se therapy was instituted[8,9]. Some of these conditions in neonates may be at least partly attributable to damage caused by oxygen radicals. These include bronchopulmonary dysplasia, retinopathy of prematurity, necrotizing enterocolitis, patent ductus arteriosus and neuronal injury in Hypoxic ischemic encephalopathy[5,6,10,21]. Symptoms of acute toxicity include nausea, vomiting, diarrhea, hair loss and exfoliative dermatitis. Se toxicity in humans has been well documented in residents of Hubei province of China, 50-80% of inhabitants developed loss of hair and nails and lesions of skin, nervous system and teeth[17].

We detected the blood Se level was decreased significantly after blood exchange transfusion; apparently this drop is related to the lower Se level of donors blood.

The effect of an exchange transfusion on antioxidants in the plasma of newborns with rh hemolytic disease was studied. Exchange transfusion rapidly produced variable changes in the concentrations of prooxidant and antioxidant substances in plasma and may thus influence free radical metabolism in the newborn[22]. There was no detectable change in mean Se or glutathione peroxidase concentration after transfusion[23]. Newborns are subject to increased oxidative stress and in cases where there is a depressed antioxidant system, which is significantly shown in cord blood and on the 4$^{th}$ day of life in babies with high bilirubinemia compared to less jaundiced babies[4].

**Table 3:** Comparison of average and standard deviation of Se (ppb) levels before and after EXT with donors' blood.

| Before. EXT Mean (SD) | Donor Mean (SD) | After EXT Mean (SD) |
|---|---|---|
| 629.78 (283.82) | 507.90 (223.56) | 454.83 (213.75) |

SD: Standard Deviation

In this study, significant correlations were observed between the blood levels of Se before EXT and total serum bilirubin levels. More comprehensive studies are needed in order to elucidate the role of Se in idiopathic unconjugated hyperbilirubinemia in neonates.

However, this is a preliminary study, possibly the first; more studies with large number of cases are required to confirm the feasibility and potency of this method as a research laboratory test. We did not study Se level in mothers; this is the limitation of the study.

## Conclusion

The blood Se level was decreased significantly after blood exchange transfusion. Apparently this drop is related to the lower Se level of donor's blood, and exchange is another factor that influences Se status in newborns. There was a significant correlation between the blood levels of Se before EXT and total serum bilirubin levels.

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## References

1. Krachler M, Rossipal E, Micetic-Turk D. Trace element transfer from the mother to the newborn-investigations on triplets of colostrums, maternal and umbilical cord sera. *Euro J Clin Nutr* 1999; 53(6):486-94.
2. Loui A, Raab A, Obladen M, et al. Nutritional zinc balance in extremely low-birth-weight infants. *J Pediatr Gastroenterol Nutr* 2001;32(4):438-42.
3. Castello AML, Osrin D. Micronutrient status during pregnancy and outcomes newborn infants in developing countries. *J Nutr* 2001;133(5 Suppl 2):1757S-64S.
4. Turgut M, Başaram O, Çekmen M, et al. Oxidant and antioxidant levels in preterm newborns with idiopathic hyperbilirubinaemia. *J Pediatr Child Health* 2004;40(11):633-7.
5. Gathwala G, Yadav OP. Se in the neonate. *Indian J Pediatr* 2002;69(5):443-6.
6. Darlow BA, Austin N. Se supplementation to prevent short-term morbidity in preterm neonates. *Cochrane Database Syst Rev* 2003;(4):CD003312.
7. Garhwali G, Yadav OP, Singh I, et al. Maternal and cord plasma Se levels in full term neonates. *Indian J Pediatr* 2000;67(10):729-31.
8. Strabo M, Longing M, Mezzos P, et al. Se status, birth weight, and breast-feeding pattern in the first month. *Biolo Trace Elem Res* 2004;99(1-3):71-81.
9. Su M, Tian D, Li W, et al. Analysis of iodine and Se trace elements in umbilical cord blood in cretinous regions in northwest China in 1999. *Environ Health Prev Med* 2002;7(1):19-21.
10. Mostafa-Gharehbaghi M, Mostafa-Gharabaghi P, Ghanbari F, et al. Determination of Se in serum samples of preterm newborn infants with bronchopulmonary dysplasia using a validated hydride generation system. *Biol Trace Elem Res* 2012;147(1-3):1-7.
11. Sağmanhgil Özdemir H, Karadas F, Pappas A, et al. The Se levels of mothers and their neonates using hair, breast milk, meconium, and maternal and umbilical cord blood in Van Basin. *Biol Trace Elem Res* 2008;122(3):206-15.
12. Filby RH. Isotopic and nuclear analytical techniques in biological systems: A critical study, Part IX. Neutron activation analysis (Technical report). *Pure Appl Chem* 1995;67(11):1929-41.
13. Bakir MA, Yaseene T, Sarheel A, et al. The determination of Se concentration in blood and tumor tissues of breast cancer patients in Syria using instrumental neutron activation analysis. *J Radioanal Nucl Chem* 2004;260(3):607-12.
14. Neutron activation analysis. Available at: http://en.wikipedia.org/wiki/neutron_activation_analysis. Access date: Aug 10, 2012.
15. Safaralizadeh R, Kardar GA, Pourpak Z, et al. Serum concentration of Se in healthy individuals living in Tehran. *Nutr J* 2005;4:32.

16. Carmona-Fonesca J. Se in serum and plasma: epidemiology and reference values. *Rev Panam Salud publica* 2010;28(5):388-98.
17. Muntau AC, Streiter M. Age-related reference values for serum Se concentrations in infants and children. *Clin Chem* 2002;48(3):555-60.
18. Safaralizadeh R, Sirjani M, Pourpak Z. Serum Se concentration in healthy children living in Tehran. *Bio Factores* 2007;31(2):127-31.
19. Aydin A, Sayal A, Işimer A. Plasma glutathione peroxidase activity and Se levels of newborns with jaundice. *Biol Trace Elem Res* 1997;58(1-2):85-90.
20. Boskabadi H, Rezagholizadeh, Omran F, et al. The effect of maternal Se supplementation on pregnancy outcome and the level of oxidative stress in neonates. *Iran Red Crescent Med J* 2010;12(3):1-6.
21. Mentro MA, Smith AM, Moyer-Miler L. Plasma and erythrocyte Se and glutathione peroxidase activity in preterm infants at risk for brochopulmonary dysplasia. *Biol Trace Elem Res* 2005;106(2):97-106.
22. Lindeman JH, Lentjes EG, Houdkamp EX. Effect of an exchange transfusion on plasma antioxidants in the newborn. *Pediatrics* 1992;90(2 Pt. 1):200-3.
23. Wilson DC, Tubman TR, Bell N, et al. Effect of blood transfusion on plasma selenium, glutathione peroxidase and Manganese levels in very low birth weight infants. *Biol Neonate* 1991;60(3-4):148-51.