Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Trace Elements in Medicine and Biology 19 (2005) 49–54
Journal of Trace Elements in Medicine and Biology
www.elsevier.de/jtemb

SECOND INTERNATIONAL FESTEM SYMPOSIUM

Selenium levels in related biological samples: Human placenta, maternal and umbilical cord blood, hair and nails

María José Lorenzo Alonso[a], Adela Bermejo Barrera[a], José Angel Cocho de Juan[b], José María Fraga Bermúdez[b], Pilar Bermejo Barrera[a,*]

[a]*Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry, University of Santiago de Compostela, Santiago de Compostela, Spain*
[b]*Unit of Metabolic and Nutritional Disorders, Department of Pediatrics, Faculty of Medicine, Hospital Clinico Universitario de Santiago, University of Santiago de Compostela, Santiago de Compostela, Spain*

Abstract

A study on selenium levels has been carried out in human placenta, maternal and umbilical cord blood, hair and nails of a group of 50 mothers and in the hair of the newborns. The determinations were perfomed by electrothermal atomic absorption spectrometry.

The selenium concentration obtained for each sample type was as follows: For the human placenta the values obtained were between 0.56 and 1.06 μg/g (mean ± standard deviation: 0.81 ± 0.02 μg/g). The levels for the umbilical cord blood were 51.1–104.2 μg/l (76.3 ± 6.5 μg/l). For the maternal blood the values measured were between 57.3 and 117.9 μg/l (90.0 ± 15.2 μg/l), and for hair and nails were 0.22–1.5 μg/g (0.60 ± 0.37 μg/g) and 0.46–1.57 μg/g (0.90 ± 0.27 μg/g), respectively. For the hair of the newborns the values obtained were between 0.40 and 2.53 μg/g (1.04 ± 0.48 μg/g).

The effect of different variables as age, habitat, nutritional index or gestation age of the mothers on the selenium concentration in the samples was studied. The influence of the habitat is significant with a confidence level of 95% for the selenium concentration in maternal blood and umbilical cord blood samples. The influence of the mothers' age is significant with a confidence level of 95% for the selenium concentration in the umbilical cord blood samples. For the placenta samples, the effect of the nutritional index is significant with a confidence level of 95%.

There is a positive correlation between samples of umbilical cord blood and the newborns' hair, between placenta and umbilical cord, and between cord blood and maternal blood.

Keywords: Selenium; Electrothermal atomic absorption spectrometry; Whole blood; Human placenta; Umbilical cord blood; Hair; Nails

Introduction

In recent years, selenium has become an important trace element studied. Its biochemistry, toxicology and nutritional importance have been reviewed regularly and thoroughly over the last decade [1]. The element is essential for mammals, including humans, as a component of two enzymes, glutathione peroxidase (GSH-Px) and iodothyronine 5′-deiodinase. Selenium deficiency has been investigated with respect to a considerable number of human illnesses; a prophylactic role of the element in cancer prevention is the subject of both intense debate and epidemiological study.

*Corresponding author.
E-mail address: pbermejo@usc.es (P. Bermejo Barrera).

0946-672X/$ - see front matter © 2005 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jtemb.2005.07.006

Selenium and its determination in biological materials may become clinically relevant in situations of excessive or insufficient intake. Except for isolated areas of China, where human selenosis has been endemic [2], selenium poisoning in humans is uncommon, but can be serious. Toxicity due to the ingestion of concentrated solutions and incorrectly formulated health supplements has been reported [3]. Classical chronic selenosis manifests as a gastro-intestinal disturbance, ''garlic-breath'', loss of hair and nails, infection of the nail beds and skin lesions. More relevant is the biochemical monitoring of selenium in patients deemed to be at risk of deficiency as a consequence of illness or therapy. Selenium deficiency is associated with cardiomyopathy, haematological abnormalities and myalgias, after 2.6 and 8 years of ''selenium free'' long-term parenteral nutrition. Further studies indicated that, although symptomatic selenium deficiency was rare, biochemical depletion occurred in the absence of supplementation [4,5].

The status of selenium is important in the group of hospitalized neonates and infants. Biological markers of selenium nutritional status [6] include the selenium concentrations in whole blood, plasma and urine that reflect the recent intake. The determination of GSH-Px activity in red blood cells and platelets gives an indirect measure over a 3-month period. The recommended daily allowance of selenium is 50–200 μg [7] and there is a significant correlation between the dietary intake of selenium and its concentration in whole blood. After absorption selenium is carried to the plasma and then is delivered to all tissues. Measurement of the selenium content in hair and nails offers a long-term marker. Hair has been shown to be a major vehicle for excretion of toxic metals and the concentration of metals in hair is up to ten-fold higher than the levels found in blood or urine samples.

In this paper, a study on the selenium levels in human placenta, maternal and umbilical cord blood, hair and nails and their possible correlation is carried out.

Material and methods

Apparatus

A Perkin-Elmer Model 4100B atomic absorption spectrometer equipped with a Zeeman effect background correction, an THGA graphite furnace atomizer and an AS-71 autosampler was used for selenium measurements in placenta and blood samples. The source of radiation was an electrodeless discharge lamp. Spectrometric operating conditions are given in Table 1.

Determinations of selenium in hair and nails were performed on a Perkin-Elmer Model 1100B atomic absorption spectrometer equipped with an HGA-700 graphite furnace atomizer and an AS-70 autosampler. The selenium electrodeless discharge lamp was operated at 6 W (selenium 196.0 nm line, spectral bandwidth 2.0 nm). Deuterium background correction was used. Pyrolytic graphite-coated tubes with L'vov platforms

Table 1. Spectrometric operating conditions and graphite furnace temperature programme for selenium determination in samples of human placenta and blood

Step	Temperature (°C)	Ramp (s)	Hold (s)	Ar flow (ml/min)
Drying	150	5	20	300
Ashing	1200	5	30	300
Atomization	2200	0	4	0
Cleaning	2300	1	4	300
Wavelength: 196.0 nm Spectral bandwidth: 2 nm Discharge lamp power: 6 W Zeeman background correction			Peak area measurements Integration time: 4 s Injection volume: 20 μl	

Table 2. Graphite furnace temperature programme for selenium determination in samples of human hair and nails

Step	Temperature (°C)	Ramp (s)	Hold (s)	Ar flow (ml/min)
Drying	150	5	20	300
Ashing	1200	5	30	300
Atomization	1900	0	4	0
Cleaning	2000	1	4	300
Wavelength: 196.0 nm Integration time: 4 s			Peak area measurements Injection volume: 20 μl	

were used. The temperature and time programmes are shown in Table 2.

Reagents

All solutions were prepared from analytical reagent grade chemicals using ultrapure water obtained from a Milli-Q system.

A 1000 mg/l selenium stock standard solution (Merck, Darmstadt, Germany) was used for the preparation of working standard solutions containing 10, 20 and 30 μg/l of selenium. A 3000 mg/l palladium solution was prepared according to Welz et al. [8] by dissolving 300 mg of palladium (99.999% purity, Aldrich, Milwakee, USA) in 1 ml of concentrated nitric acid and diluting to 100 ml with ultrapure water. Other reagents used were: concentrated nitric acid (69–70%, Scharlau, Barcelona, Spain), hydrogen peroxide (30%, Panreac, Montcada y Reixac, Barcelona, Spain).

Reference materials used were:

- Seronorm Trace Elements, Whole Blood 404107 (Nycomed AS, Oslo, Norway) with a certified selenium content of 80 (80–91) μg/l.
- Seronorm Trace Elements, Whole Blood 404108 (Nycomed AS) with a certified selenium content of 82 (82–89) μg/l.
- Seronorm Trace Elements, Whole Blood 404109 (Nycomed AS) with a certified selenium content of 79 (79–84) μg/l.
- Reference material NIST 1577a (Bovine Liver) obtained from the National Institute of Standard Technology with a selenium content of 0.7 ± 0.07 μg/g.
- Reference material IAEA H-8 obtained from the International Atomic Energy Agency with a selenium content of 4.67 ± 0.96 μg/g.
- Reference material BCR 186 (Pig Kidney) obtained from the Commission of the European Communities, Community Bureau of Reference with a selenium content of 10.3 ± 0.5 μg/g.
- Reference material CRM 397 (Human Hair) from the Commission of the European Communities, Community Bureau of Reference.

Human placenta

A method for the selenium determination in human placenta using electrothermal atomic absorption spectrometry (ETAAS) with Zeeman effect background correction was developed.

Placenta sampling and treatment

The human placenta samples were collected at the University Clinic Hospital of Santiago de Compostela by authorized personnel in plastic vessels and kept at –20 °C. Later the samples were dried in an oven at 100–110 °C, pulverized in a porcelain mortar and stored in plastic vessels until use.

The human placenta samples were decomposed by acid digestion in a microwave to convert organic compounds of selenium to inorganic selenium. The digestion was carried out in PTFE vessels as follows: 0.1 g of dried human placenta sample was weighted and 1 ml of nitric acid was added directly into a vessel. The vessel was then capped and heated 6 min at 294 W in the microwave. After cooling in an ice bath, the vessel was opened and 0.3 ml of hydrogen peroxide was added. The vessel was resealed and heated 6 min in the microwave. After cooling, the digestion mixture was diluted by adding up to 5 ml. A portion of 240 μl was transferred to an autosampler cup together with the appropiate amount of matrix modifier and diluted to 400 μl.

Selenium determination

The atomic absorption measurements were performed at 196.0 nm with a Zeeman background correction system. A volume of 20 μl of the sample solution was injected and the temperature programme was optimized (Table 1).

All the measurements were carried out in the presence of chemical modifier, optimizing its concentration once the heating programmes had been selected. The linearity of the method was studied by comparing the slopes of the calibration straight line for aqueous solutions and for the standard additions method. The slopes were not similar for a 95% significance level, and the calibration was carried out using the standard addition method.

The accuracy of the method was confirmed by studying three reference materials with a certified selenium content of 0.7 ± 0.07, 4.67 ± 0.96, 10.3 ± 0.5 μg/g, respectively. There was good agreement between the reference values and the results. The sensitivity was calculated by limit of detection, limit of quantification and characteristic mass.

Maternal and umbilical cord blood

A method for the selenium determination in acid digested whole blood samples was developed using ETAAS. In the selected experimental conditions, Zeeman background correction was used to compensate the high background signal. The procedure was applied for the selenium determination in 48 samples of maternal blood and the corresponding umbilical cord blood.

Blood sampling and treatment

Blood samples were taken from the volunteers at the University Clinic Hospital of Santiago de Compostela

by authorized personnel. Blood was heparinized and samples taken were stored in a refrigerator at 4 °C.

Blood samples were decomposed by acid digestion in a microwave. The digestion was carried out in PTFE vessels as follows: 1 ml of blood sample was measured and 1 ml of nitric acid and 0.3 ml of hydrogen peroxide were added directly into the vessel. The vessel was then capped and heated 6 min at 294 W in the microwave. After cooling in an ice bath, the vessel was opened and the mixture was diluted by adding up to 5 ml. A portion of 200 μl was transferred to an autosampler cup together with the appropriate amount of matrix modifier and diluted to 400 μl.

Selenium determination

The atomic absorption measurements were performed at 196.0 nm with a Zeeman background correction system. A volume of 20 μl of the sample solution was injected into the atomizer and the drying–ashing–atomization–cleaning programme of the graphite furnace (Table 1) was run and the integrated absorbance recorded.

All the measurements were carried out in the presence of chemical modifier, optimizing its concentration once the heating programmes had been selected. Palladium was used as a matrix modifier.

Calibration was carried out using the standard addition method. The detection limit was 11.2 μg/l. The reliability of the procedure was checked by analyzing three certified reference materials and by recovery studies.

Hair

A method for the selenium determination in the hair of the mothers and their children was optimized.

Hair sampling and treatment

Approximately, 0.5 g of hair was cut with stainless-steel scissors from the scalp region, the hair length varied between 1 and 3 cm. Washing of the hair samples was required to provide an accurate assessment of the endogenous metal content. The washing procedure was carried out according to the recommendations of the IAEA [9]: Hair samples were first washed with ultrapure water, then washed three times with acetone, and finally they were again washed with ultrapure water. The samples were then oven-dried at 100 °C.

An acid digestion procedure in a microwave was optimized. The acid digestion was carried out in a domestic microwave oven with laboratory made low pressure PTFE bombs. In total, 0.1 g of hair sample was weighed in the PTFE bombs and 1 ml of nitric acid was added and the sample heated in the microwave at 330 W for 6 min. Then 0.3 ml hydrogen peroxide was added and the samples were heated in the microwave for another 6 min. Finally, the acid digests were made up to 5 ml with ultrapure water.

Selenium determination

The samples were measured by ETAAS with deuterium background correction.

To achieve complete mineralization, the samples were ashed at 1200 °C in the presence of palladium as a chemical modifier. The optimum atomization temperature was 1900 °C (Table 2). The precision and accuracy of the method were studied using the reference material CRM 397. Results of calibration using aqueous standards and the standard addition method were compared.

Results

A study on the selenium levels of human placenta, maternal and umbilical cord blood, hair and nails has been carried out in a group of 50 mothers and in the hair of the newborns. All samples were analyzed at least by triplicate.

For human placenta, the selenium values obtained are shown in Fig. 1. The results were between 0.56 and 1.06 μg/g (mean ± standard deviation: 0.81 ± 0.02 μg/g). In total, 30% of the samples presented a selenium concentration of 0.8–0.9 μg/g, 22% of the samples values of 0.7–0.8 μg/g and 4% of the samples had selenium concentrations between 1.0 and 1.1 μg/g.

The selenium levels in umbilical cord were between 0.20 and 0.45 μg/g with a mean value of 0.27 ± 0.05 μg/g. In total, 50% of the samples presented values of 0.2–0.3 μg/g and only 8% of samples had concentrations between 0.40 and 0.50 μg/g.

The selenium levels in maternal blood samples are shown in Fig. 2. The values obtained were between 57.3 and 117.9 μg/l (90.0 ± 15.2 μg/l). A total of 34% of the samples presented selenium concentrations between 80 and 90 μg/l, 22% of the samples had selenium values of

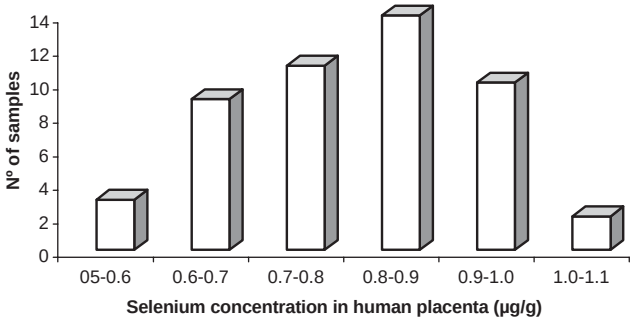

Fig. 1. Selenium concentration distribution in samples of human placenta.

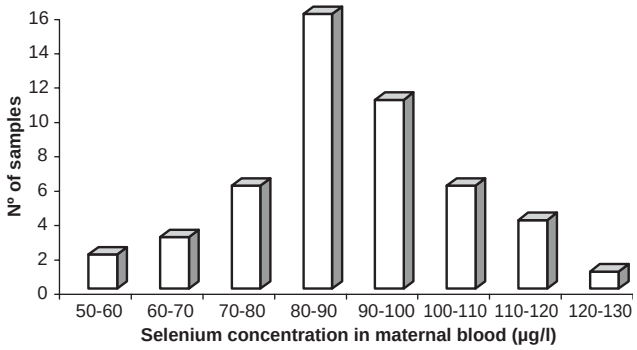

Fig. 2. Selenium concentration distribution in samples of maternal blood.

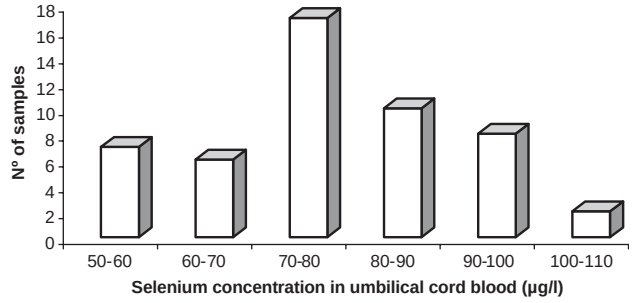

Fig. 3. Selenium concentration distribution in samples of umbilical cord blood.

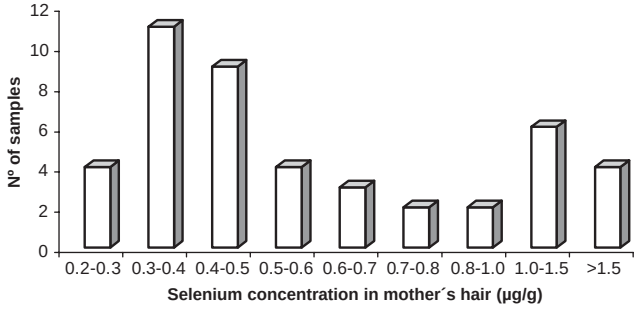

Fig. 4. Selenium concentration distribution in samples of the mothers' hair.

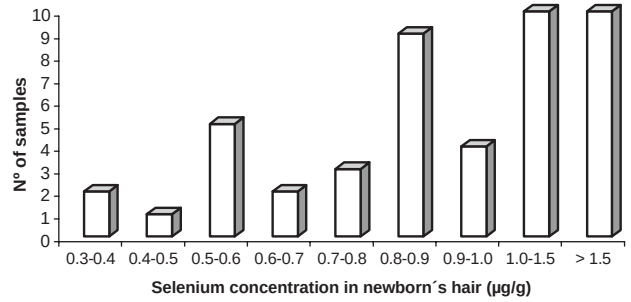

Fig. 5. Selenium concentration distribution in samples of the newborns' hair.

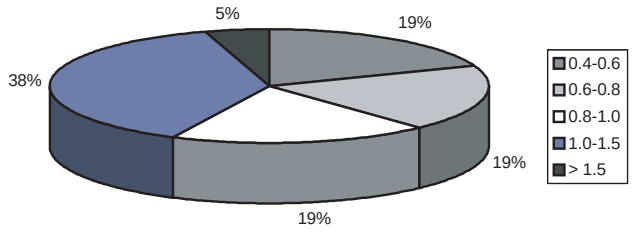

Fig. 6. Selenium concentration distribution in samples of the mothers' nails.

90–100 µg/l, only 2% of the samples presented selenium concentrations between 120 and 130 µg/l.

The distribution of selenium concentration in umbilical cord blood samples are shown in Fig. 3. The selenium levels for the umbilical cord blood samples were between 51.1 and 104.2 µg/l (76.3 ± 6.5 µg/l). A total of 34% of the samples presented selenium concentrations between 70 and 80 µg/l, 20% of the samples had values between 80% and 90%. A total of 4% of the samples presented selenium concentrations of 100–110 µg/l.

The levels of selenium for the hair of the mothers (Fig. 4) were 0.22–1.5 µg/g (0.60 ± 0.37 µg/g). The distribution shows 29% of the samples with concentrations between 0.4 and 0.5 µg/g, 15% of the samples with values of 0.5–0.7 µg/g, 4% of the samples with selenium concentrations of 0.7–0.8 µg/g and another 4% with values of 0.8–1.0 µg/g.

The results for the hair of the newborns (Fig. 5) were between 0.40 and 2.53 µg/g (1.04 ± 0.48 µg/g). In total, 22% of the samples presented selenium concentrations higher than 0.8–0.9 µg/g, followed by 21% of the samples with values between 1.0 and 1.5 µg/g. In total, 20% of the samples had selenium concentrations between 0.4 and 0.5 µg/g.

The selenium concentration distribution in the mothers' nail samples is shown in Fig. 6. The values obtained were between 0.46 and 1.57 µg/g (0.90 ± 0.27 µg/g). In total, 38% of the samples presented selenium concentrations of 1.0–1.5 µg/g, followed by 19% of the samples with values between 0.8 and 1.0 µg/g. A low percentage of the samples, 5%, presented selenium concentrations higher than 1.5 µg/g.

Discussion and conclusion

The effect of different variables – gestation age, habitat, age of the mothers and nutritional index – on the selenium concentration in maternal blood, umbilical cord blood and placenta has been studied.

The gestation age was divided in <37 and >37 weeks. There is no significant influence of this variable; there are no differences for a confidence level of 95% in the selenium concentration in the different samples.

The habitat was divided into rural, coastal and urban. The influence of the habitat was significant with a confidence level of 95% for the selenium concentration in samples of maternal blood an umbilical cord blood, but was not significant for the human placenta. The selenium concentrations were lower in samples from rural habitat.

The age of the mothers was considered in intervals of <32 and >32 years. The influence of the mothers' age was significant with a confidence level of 95% for the selenium concentration in samples of umbilical cord blood, with lower levels for the <32-year group.

The nutritional index was divided into <0.3 and >0.3. The influence of the nutritional index was significant with a confidence level of 95% for the selenium concentration in placenta samples, with lower levels for the >0.3 group.

A study on the possible correlation between the different samples studied has been carried out. There is a positive correlation with a regression coefficient of 0.46 between the cord blood and the newborns' hair. Moreover, there is a positive correlation between the levels of selenium in the placenta and in umbilical cord with a regression coefficient of 0.67. Furthermore, for the maternal blood and cord blood there is also a positive correlation with a regression coefficient of 0.78.

We can conclude that the status of selenium in the newborn depends on the selenium status of the mother because low levels of this element in the mother produce low levels in the cord blood.

References

[1] Lockitch G. Selenium: clinical significance and analytical concepts. Crit. Rev. Clin. Lab. Sci. 1989;27:483–541.
[2] Yang G, Wang S, Zhor R, Sun S. Endemic selenium intoxication of humans in China. Am. J. Clin. Nutr. 1983;37:872–81.
[3] Clark RF, Strukle E, Williams SR, Manoguerra AS. Selenium poisoning from a nutritional supplement. J. Am. Med. Assoc. 1996;275:1087–8.
[4] Lane HW, Barroso AO, Englert D, Dudrick SJ, MacFadden BS. Selenium status of seven chronic intavenous hyperalimentation patients. J. Parent. Ent. Nutr. 1982;6:426–31.
[5] Shenkin A, Fell GS, Halls DJ, Dunbar PM, Holbrook IB, Irving MH. Essential trace element provision to patients receiving home intravenous nutrition in the UK. Clin. Nutr. 1986;5:91–7.
[6] Neve J. In: Neve J, Favier A, editors. Selenium in medicine and biology. Berlin: Walter de Gruyter; 1988. p. 137.
[7] Bermejo-Barrera P, Moreda-Piñeiro A, Moreda-Piñeiro J, Bermejo-Barrera A. Slurry sampling – electrothermal atomic absorption spectrometric determination of lead, cadmiun and manganese in human hair samples using rapid atomizer programs. J. Anal. At. Spectrom. 1997;12:1–6.
[8] Welz B, Schlemmer G, Madakavi JR. J. Anal. At. Spectrom. 1988;3:395.
[9] Report on the Second Research Co-ordination Meeting of IAEA, Neuherberg, 1985.