Assessing reference levels of nickel and chromium in cord blood, maternal blood and placenta specimens from Ankara, Turkey

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Abstract

Objective: Placenta is a temporary organ that connects the developing fetus and the mother. However, it cannot protect the embryo against chromium (Cr) and nickel (Ni) exposure. Quantification of Cr and Ni in biological and ecological subjects is challenging. Thus, the first goal of this study was to provide a validated Graphite Furnace Atomic Absorption Spectrometry (GFAAS) method to determine Cr and Ni in mother-newborn specimens. The second goal was to assess the reference Ni and Cr contents in cord blood, maternal blood, and placenta samples in a population from Ankara.

Material and Methods: Biological samples were collected from 100 healthy mother-newborn pairs. Metal levels were quantified by GFAAS. Method validation of this toxicological analysis was performed by the use of certified reference materials, and assessed through accuracy, precision, specificity, range, quantitation, and detection limits.

Results: Mean Cr levels of maternal blood, placentas, and cord blood were 0.337±0.222 μg/L, 0.221±0.160 μg/kg, 0.121±0.096 μg/L, respectively while mean Ni concentrations were 0.128±0.093 μg/L, 0.124±0.067 μg/kg, 0.099±0.067 μg/L, respectively. The method showed linearity with excellent correlation coefficients (r^2) for Cr (0.9994) and Ni (0.9999). Satisfactory recovery and coefficient of variation for Cr and Ni were 102.85% and 102.35%; 1.75% and 2.91%, respectively. Relative error did not exceed 3%, demonstrating the accuracy of the method. Control charts were drawn to assess inter-day stability. The predicted reference ranges for Cr and Ni concentrations in maternal blood, placenta and cord blood were: Cr 0.033-0.75 μg/L; 0.032-0.526 μg/kg; 0.031-0.309 μg/L and for Ni were 0.011-0.308 μg/L; 0.024-0.251 μg/kg; 0.066-0.209 μg/L, respectively.

Conclusion: The reported reference values of biological specimens in this paper will provide complementary aid to health professionals in terms of assessment of environmental and occupational exposure. (J Turk Ger Gynecol Assoc 2021; 22: 187-95)

Keywords: Placenta, cord blood, maternal blood, nickel, chromium, validation, reference range, GFAAS

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Introduction

Certain compounds of chromium (Cr) and nickel (Ni) are poisonous, particularly with increasing long-term exposure. For instance, nickel carbonyl or hexavalent chromium are categorized as carcinogens. Human exposure to Cr and Ni can arise via ingestion of polluted water or food, as well as inhalation or dermal contact, since these metals are applied in an elemental form in many industrial activities (1). There are also additional paths of exposure to Cr and Ni such as smoking or contact with coins, stainless steel and jewelry (2,3), particularly in the daily life of pregnant women.

Chromium-related ecological pollution has been increasing as a result of its greater worldwide industrial usage. Exposure to chromium can cause critical medical disorders, such as abnormal enzymatic activity, oxidation-reduction derangement and protein denaturation. In addition, asthma,

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back pain, dermatitis, cancer, chromosomal aberrations, chronic bronchitis, changes in hemoglobin, hypertension and metabolic syndrome have all been associated with chromium exposure (4,5). Chromium can cross the placenta (6). Previous animal studies have suggested that exposure to elevated chromium levels in the prenatal period harms implantation and embryonic growth (7). Furthermore, there is evidence that fetal resorption, intrauterine death, skeletal anomalies, decreased fetal weight, malformations and retarded fetal growth may be associated with chromium exposure (8). Most of the present information regarding the health effect of exposure to chromium depends on data obtained following occupational exposure. Nevertheless, several studies have suggested an increased risk of inborn abnormalities, reduced birth weight and DNA damage in neonates born into areas affected by chromium pollution (9,10).

Nickel also crosses the placenta and has been shown to impair fetal development in animal studies (11). Numerous investigations demonstrate that heavy metal contamination is a changeable risk issue in terms of perinatal results along with many congenital disorders (12). The relationship between cancer and Ni depends on industrial exposure and has been associated with different types of cancer including kidney, stomach, breast, and neck/head and nose malignancies (13). Additionally, exposure to high concentrations of Ni may lead to contact dermatitis, epigenetic changes, alteration in gene regulation and apoptosis induction (14). Also, exposure to Ni may cause developmental and reproductive toxicological effects, which include birth defects, abortion, fertility or subfertility (15,16). Moreover, embryonic progression, a declining proliferation of inner cell mass and trophoblast cells may be influenced by exposure to excess amounts of Ni (17). This evidence suggests that exposure to Ni is a critical problem, both for public health and environmental protection (18,19).

Since there is limited knowledge regarding the effect of Cr and Ni exposure in the population in terms of prenatal development, supplementary studies investigating placental transfer of these heavy metals are required (20). In addition, analysis of chromium and nickel in biological and ecological samples is not easy due to interferences in the matrix and possible low levels in specimens (21). Therefore, sensitive methods to quantify Cr and Ni in biological and environmental specimens has become a critical research topic for public health. Thus, an extremely sensitive and sophisticated analytical assay is necessary.

To the best of our knowledge, there is no study focusing on toxicological monitoring of Cr and Ni profiles in maternal blood, placenta and cord blood in the Turkish population. From this point of view, the overall objectives of this investigation were twofold. The first goal was to optimize and validate the Graphite Furnace Atomic Absorption Spectrometry (GFAAS) methods to quantify Cr and Ni concentrations at trace levels in biological samples. The second target was to provide toxicological monitoring of Cr and Ni profiles in maternal blood, cord blood and placenta samples in the Turkish population, thus providing a measure of possible environmental exposure, as well as providing reference values of chromium and nickel in these biological tissues.

Material and Methods

Study subjects

This scientific work was ethically authorized by the Research Ethics Committee of the Ankara University Faculty of Medicine (approval number: 33-730/July 11, 2011). Each volunteer provided written informed consent in line with the ethics as recognized in the Declaration of Helsinki (World Medical Association, Declaration of Helsinki, 1964).

All research samples were collected in Ankara, Turkey as the capital of Turkey experiences relatively light exposure to industrial pollution. Participants having no known industrial or environmental exposure to xenobiotics, including heavy metals, were included based on the sample selection criteria. Of 137 eligible participants, some were excluded due to improperly completed consent forms while several had a diagnosis of intrauterine growth retardation. Hence, 100 healthy mother-newborn pairs were recruited to the study. The final cohort consisted of mothers aged 19-41 years who had given birth at between 36-41 weeks gestation. Placenta, cord blood, and maternal blood samples were gathered at delivery by cesarean section or spontaneous labor. Blood specimens were collected into vacutainer blood tubes and stored at 4 °C, while placenta samples were kept at -20 °C until the day of analysis.

Standard solutions and reagents

Stock solutions of 1000 µg/mL Cr and Ni were purchased from SCP Science AA Standards (Canada). Nitric acid (HNO_3, 65% v:v) was procured from Merck (Darmstadt, Germany). The chemicals used for the laboratory work were at analytical reagent grade. High purity (99.999%) argon gas was bought from a local supplier (Vasak Gaz, Ankara, Turkey). With the resistivity of 18MΩ cm, ultrapure water (Merck Millipore Direct-Q8, Germany) was utilized to prepare the solutions for the experimental study. The certified reference material (CRM) NC SZC 73016-Chicken (NCS Testing Technology Co., Ltd., Haidian, Beijing, China) was used for validation of the method.

Sample preparation and procedure

To prepare calibration standards at concentrations of 0.5, 1.0, 5.0, 10.0, and 20.0 mg/L, a 1000-µg/mL of Cr and Ni stock solution were diluted with 10% (v:v) HNO_3. A relatively

high concentration of nitric acid was used in our calibration standards to simulate the acid content in the final digested biological samples. A previously described digestion protocol was followed (22-24) before starting the instrumental analysis. One milliliter volumes of blood samples and accurately weighed dry placenta samples (not exceeding 200 mg) were liquified with 5 mL of 65% (v/v) nitric acid in Teflon® microwave tubes. Digestion was carried out at 1600 W and 220 °C for 20 minutes by means of the microwave system Mars Xpress (CEM, Matthews, NC, USA). Then the liquified solutions were diluted in ultra-pure water to 10 mL in 15 mL polypropylene tubes. The samples were kept at 4 °C until the day of analysis.

Instrumentation

Cr and Ni levels in maternal blood, cord blood, and placenta samples were quantified using a Varian AA 240 GFAAS with Zeeman background correction (Varian Corp, Victoria, Australia). Boosted-discharge hollow cathode lamps (Agilent, USA) were utilized as the excitation source for Cr and Ni. The instrumental working parameters for the GFAAS system were shown in Table 1.

Table 1. Operating parameters of GFAAS method

Element - matrix	Cr-Blood/ Placenta	Ni-Blood/ Placenta
Instrument	Zeeman	Zeeman
Concentration unit	µg/L; µg/kg	µg/L; µg/kg
Instrument mode	Absorbance	Absorbance
Sampling	Auto-mix	Auto-mix
Calibration mode	Concentration	Concentration
Measurement mode	Peak height	Peak height
Replicates standard	3	3
Replicate sample	3	3
Expansion factor	1.0	1.0
Wavelength	357.9 nm	232.0 nm
Slit width	0.2 nm	0.2 nm
Gain	45%	75%
Current	15.0 mA	4.0 mA
Background	BC on	BC on
Standard 1	0.5 µg/L	0.5 µg/L
Standard 2	1.0 µg/L	1.0 µg/L
Standard 3	5.0 µg/L	5.0 µg/L
Standard 4	10.0 µg/L	10.0 µg/L
Standard 5	20.0 µg/L	20.0 µg/L
Reslope standard	Standard 3	Standard 3
Recalibration rate	50	50
Calibration algorithm	Linear	Linear

GFAAS: Graphite Furnace Atomic Absorption Spectrometry, Cr: Chromium, Ni: Nickel, BC: Background

Statistical analysis

The use of various statistical methods assessed the elemental quantifications in mother-newborn biological specimens. The Kolmogorov-Smirnov test was utilized for assessment of normality of data distribution while correlations between the parameters were examined through the Pearson's test. Statistical significances among mean values were evaluated employing the Student's t-test. Statistical test results were interpreted as mean ± standard deviation (SD) of the mean. Statistical significance was assumed when $p<0.05$. SPSS® software version 16.0 was used throughout the statistical analysis.

Results

Optimization

In order to achieve the best possible performance, this method was optimized in terms of digestion technique, appropriate wavelength for the placenta and blood matrix, calibration concentration range in keeping with the Cr and Ni concentration in real biological specimens, and approximating linearity as much as possible.

Absorbance was quantified as a function of Cr and Ni concentration at 357.9 nm, and 232.0 nm, respectively. The proposed methods show good linearity in the range of 0-20 µg/L for Cr and Ni. The correlation coefficients and equation of the calibration curves for Cr and Ni were respectively found to be r^2: 0.9994 Abs: 0.0384C+0.0044 and r^2: 0.9999 Abs: 0.0071C+0.0003, where Abs is integrated absorbance and C is the concentration in µg/L. Graphite furnace temperature programs for Cr and Ni are listed in Table 2.

Validation

In keeping with the validation guide ISO/IEC 17025 standard (25) method, validation of this toxicological assay was performed by use of CRM, which was resulted in calculation of the accuracy, precision, specificity, range, quantitation and detection limits. CRM was analyzed 11 times with triplicate measurements. The results were compared to the certified values to evaluate the accuracy, precision, and recovery of the method. The certified Cr content of the CRM was 590.00±11.00 µg/kg, while the measured value was 606.84±10.65 µg/kg with the successful percent recovery and coefficient of variation (CV) of 102.85% and 1.75%, respectively. Similarly, the certified Ni content of the CRM was 150.00±3.00 µg/kg, while the measured value was 153.53±4.47 µg/kg, with the successful percent recovery and CV of 102.35% and 2.91%, respectively. Relative error did not exceed 3%, indicating that the method was accurate. The validation study of this assay is summarized in Table 3.

The limit of detection (LOD) and lowest limit of quantification (LOQ) was computed utilizing the SD of the response and

the slope of the calibration curve, according to ICH guiding principle (26) (LOQ: 10σ/S, LOD: 3.3σ/S, where S is the slope of the calibration curve and σ is the SD of the response). GFAAS method for Cr and Ni analysis provided detection and quantification limits of 0.010 and 0.030 and 0.060 and 0.182, respectively.

Quality control

The control chart analysis offers an examination of the inter-day and intra-day stability of the instrument (27-29). In other words, control charts make available tracking the accuracy of routine analytical work (30). Therefore, a mixture solution containing Cr and Ni at a concentration of 100 µg/L was quantified by GFAAS assay once a day throughout two weeks, and the mean concentrations of Cr and Ni were quantified as 100.18±2.09 µg/L, and 100.05±2.21 µg/L, respectively. Next, warning limits were computed by the following formula: warning limits: $x_{mean} \pm 2\sigma$, while control limits were quantified from the formula: control limits: $x_{mean} \pm 3\sigma$. Thus, the lowest control limit, upper control limit, lowest warning limit and upper warning limit were calculated accordingly for Cr and Ni. The results of the control chart study for Cr and Ni are shown in Figure 1, 2, indicating that the inter-day stability of the instrument was acceptable.

Data analysis

The outcomes of this toxicological investigation were statistically analyzed with the SPSS, version 16.0 (IBM Inc., Armonk, NY, USA). Descriptive statistics for Cr and Ni analyses in maternal blood, placenta and cord blood are shown in Table 4. Mean Cr levels of maternal blood, placenta samples, and cord blood were 0.337±0.222 µg/L, 0.221±0.160 µg/kg, 0.121±0.096 µg/L, respectively. Similarly, mean Ni concentrations of these biological specimens were 0.128±0.093 µg/L 0.124±0.066 µg/kg, 0.099±0.067 µg/L, respectively. Hence, a statistically significant negative correlation was found between the maternal blood-Cr and cord blood-Cr levels ($r=-0.21$, $p<0.05$) while another negative correlation was determined between the placental nickel and maternal blood nickel concentrations ($r=-0.27$; $p<0.001$).

Table 2. Graphite furnace temperature programs

	Step	Temperature (°C)	Time (s)	Flow (L/min)	Signal collection		Reading	
Chromium	1	85	5.0	0.3	×	No	×	No
	2	95	40.0	0.3	×	No	×	No
	3	120	10.0	0.3	×	No	×	No
	4	900	5.0	0.3	×	No	×	No
	5	900	1.0	0.3	×	No	×	No
	6	900	2.0	0.3	×	No	×	No
	7	200	7.8	0.3	√	Yes	×	No
	8	200	2.0	0.0	√	Yes	√	Yes
	9	2550	1.1	0.0	√	Yes	√	Yes
	10	2550	2.0	0.0	√	Yes	√	Yes
	11	2550	2.0	0.3	√	Yes	×	No
Nickel	1	85	5.0	0.3	×	No	×	No
	2	95	40.0	0.3	×	No	×	No
	3	120	10.0	0.3	×	No	×	No
	4	800	5.0	0.3	×	No	×	No
	5	800	1.0	0.3	×	No	×	No
	6	800	2.0	0.0	×	No	√	Yes
	7	2400	0.8	0.0	√	Yes	√	Yes
	8	2400	2.0	0.0	√	Yes	√	Yes
	9	2400	2.0	0.3	×	No	√	Yes

Table 3. Analysis of certified reference material (NCSZC3016)

	n	Certified value[a] (µg/kg)	Measured Value[a] (µg/kg)	CV%[b]	RE%[c]	R%[d]
Cr	11	590.00±11.00	606.84±10.65	1.75	2.85	102.85
Ni	11	150.00±3.00	153.53±4.47	2.91	2.35	102.35

[a]: Values are expressed as mean ± standard deviation, [b]: Coefficient of variation, [c]: Relative error, [d]: Recovery, Cr: Chromium, Ni: Nickel, CV: Coefficient of variation

Discussion

Human toxicological biomonitoring is a unique technique to screen public health in the event of chemical exposure. Thus, an explanation of toxicological monitoring data can be utilized for health risk assessment in the presence of chemical exposure (31,32). Measurement and description of toxic substances in biological specimens of healthy normal mothers and newborns provides impartial information of general population exposure (33). The prenatal period is critical since chemical exposure to toxins may result in biological alteration (34). Estimating the reference values in biological specimens provides complementary data for health professionals in terms of assessment of environmental and occupational exposure. Therefore, toxicological monitoring of Ni and Cr before and during pregnancy has become important because of the health effects on embryos, birth defects, growth retardation and neurodevelopmental disorders.

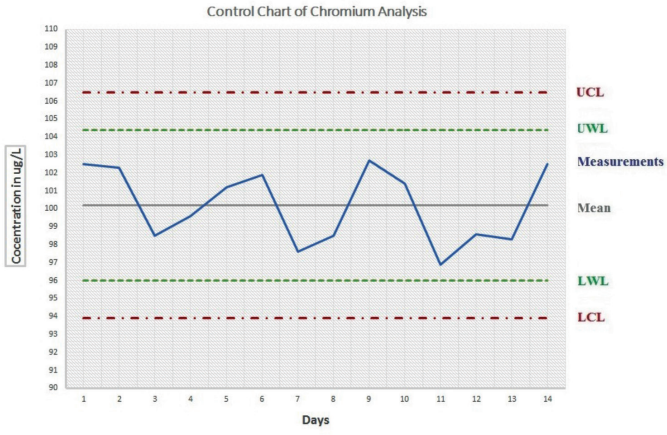

Figure 1. Control Charts of Chromium by GFAAS. The concentration of chromium is presented in ppb while UCL, UWL, LWL and LCL stand for upper control limit, upper warning limit, lowest warning limit and lowest control limit, respectively. The blue line represents the stability in the chromium concentrations quantified among days

GFAAS: Graphite Furnace Atomic Absorption Spectrometry, UCL: Upper control limit, UWL: Upper warning limit, LWL: Lowest warning limit, LCL: Lowest control limit

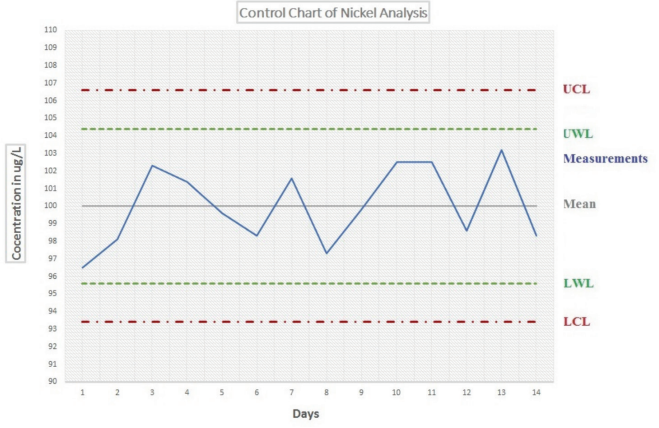

Figure 2. Control charts of nickel by GFAAS. The concentration of nickel is presented in ppb while UCL, UWL, LWL and LCL stand for upper control limit, upper warning limit, lowest warning limit and lowest control limit, respectively. The blue line represents the stability in the nickel concentrations quantified among days

GFAAS: Graphite Furnace Atomic Absorption Spectrometry, UCL: Upper control limit, UWL: Upper warning limit, LWL: Lowest warning limit, LCL: Lowest control limit

As can be seen in Table 4, mean concentrations of chromium levels in maternal blood, placenta samples and cord blood were significantly higher than nickel levels in this cohort ($p<0.05$). This finding is consistent with previous research in which blood Cr and Ni levels were studied (35). On the contrary, there are also studies reporting Ni levels are higher than Cr in biological specimens (Table 5). This may be due to the characteristics of the exposure source. Goullé et al.

Table 4. Descriptive statistics of Cr and Ni levels in biological specimens

	n		Cr[a]	Ni[a]	p value[b]
Maternal blood	100	Minimum	0.033	0.011	0.03
		Maximum	3.077	0.800	
		Mean[c]	0.337±0.222	0.128±0.093	
		5% Trimmed mean	0.306	0.122	
		Reference range	0.033-0.750	0.011-0.308	
Placenta	100	Minimum	0.032	0.024	0.04
		Maximum	0.914	0.683	
		Mean[c]	0.221±0.160	0.124±0.066	
		5% Trimmed mean	0.206	0.119	
		Reference range	0.032-0.526	0.024-0.251	
Cord blood	100	Minimum	0.031	0.066	0.04
		Maximum	1.202	0.588	
		Mean[c]	0.121±0.096	0.099±0.067	
		5% Trimmed mean	0.117	0.105	
		Reference range	0.031-0.309	0.066-0.209	

[a]: Values are given µg/L for blood samples while µg/kg for the placenta, [b]: p-values reported here indicate those mean concentrations of chromium levels in biological specimens were found significantly higher than nickel levels in these biological specimens since it is <0.05, [c]: Mean values are expressed as mean ± standard deviation, Cr: Chromium, Ni: Nickel

(36) reported reference Cr and Ni levels for blood samples of 99 healthy children as 0.49-1.86 μg/L and 0.68-2.62 μg/L, respectively. Therefore, Cr and Ni contents in biological specimens from our present study were comparatively lower than the formerly reported reference ranges, indicating that our results seem to be at safe levels. Based on previous papers (37,38), our reference values estimated in Table 4 were computed by means of the 5% trimmed mean ± 2σ, so as to lessen the impact of the skew in all paths. Since the mean -2σ value emerged as continuously inferior to the minimum value of the experimental measurements, this low-end value was involved in the reference range. Consequently, the predicted reference ranges for Cr and Ni content in maternal blood, placenta and cord blood were as follows: Cr 0.033-0.75 μg/L; 0.032-0.526 μg/kg; 0.031-0.309 μg/L and Ni 0.011-0.308 μg/L; 0.024-0.251 μg/kg; 0.066-0.209 μg/L, respectively.

Further comparison of Cr and Ni content in various matrices among the previous reports and our present study are summarized in Table 5. Zhang et al. (2) investigated the relationship between Ni exposure and the occurrence of congenital heart defects. The outcome proposed that the frequency of congenital heart defects is conceivably linked with Ni exposure (2). Novak et al. (18) showed that women with metal-on-metal implants and their children have higher cobalt and Cr levels than controls, indicating that the placenta is to some degree permeable to metal ion transport. Manduca et al. (39) researched the effect of war on metal levels in maternal hair. Their results showed that war in Gaza, as environmental exposure, elevated the metal levels including Cr and Ni levels in maternal hair. Pan et al. (10) investigated the relationship between ecological Cr exposure and premature labor in the general population. Their results indicated a potential association between the risk of delivering preterm infants and elevated exposure to Cr throughout the pregnancy (10). Callan et al. (40) performed a study highlighting maternal exposure to metals, including Cr and Ni levels in maternal blood. In Spain, Bocca et al. (41) predicted the gestational exposure to essential and toxic metals by determining their levels in maternal blood, cord blood and maternal urine. Their study suggested

Table 5. Comparison of chromium and nickel contents in various matrices among previous studies and present report

Country	Tissue	Metal	Value [a]	References
USA	Maternal serum (implant group)	Cr	1.870	(2)
USA	Infant serum	Cr	0.288	(2)
USA	Maternal serum (implant group)	Ni	0.136	(2)
USA	Infant serum	Ni	0.304	(2)
China	Maternal urine	Cr	1.01 [b]	(10)
China	Placenta (case group)	Ni	178	(18)
China	Placenta (control)	Ni	148	(18)
Palestine	Maternal hair (military attack)	Cr	2930	(39)
Palestine	Maternal hair (military attack)	Ni	2760	(39)
Australia	Maternal urine (non-smoking)	Cr	0.53	(40)
Australia	Maternal urine (non-smoking)	Ni	4.7	(40)
Australia	Maternal blood (non-smoking)	Cr	3.15	(40)
Australia	Maternal blood (non-smoking)	Ni	11.7	(40)
Spain	Cord blood	Cr	0.6	(41)
Spain	Maternal blood	Cr	0.5	(41)
China	Maternal blood	Cr	6.36	(42)
China	Cord blood	Cr	12.6	(42)
China	Maternal blood	Ni	14.5	(42)
China	Cord blood	Ni	6.1	(42)
China	Placenta (e-waste recycling town)	Cr	234.31 [b]	(43)
China	Placenta (control)	Cr	228.40 [b]	(43)
China	Placenta (e-waste recycling town)	Ni	7.64 [b]	(43)
China	Placenta (control)	Ni	14.30 [b]	(43)
China	Maternal blood	Cr	0.98	(44)
China	Maternal blood	Ni	1.81	(44)

Table 5. Continued

Country	Tissue	Metal	Value [a]	References
UK	Maternal blood	Cr	1.28	(45)
UK	Umbilical cord blood	Cr	0.378	(45)
UK	Maternal blood (control)	Cr	0.199	(45)
China	Maternal urine	Cr	2.69 [b]	(46)
USA	Placenta	Ni	111.0	(47)
Turkey	Maternal blood	Cr	0.337	Present study
Turkey	Cord blood	Cr	0.121	Present study
Turkey	Placenta	Cr	0.221	Present study
Turkey	Maternal blood	Ni	0.128	Present study
Turkey	Cord blood	Ni	0.099	Present study
Turkey	Placenta	Ni	0.124	Present study

[a]: Values are given μg/L for blood and urine samples while μg/kg for placenta and hair, [b]: Median, Cr: Chromium, Ni: Nickel, USA: United States of America, UK: United Kingdom

that metabolic and physiological variations throughout pregnancy changed the content of essential and toxic metals (41). Li et al. (42) determined the impact of heavy metals including Cr and Ni exposure during pregnancy in Beijing, China. The authors stated that there was neither a significant relationship between birth length/weight and toxic metal nor a possible issue in terms of neonatal developmental toxicity (42).

As was shown in the statistical analysis, this study highlighted a statistically significant negative correlation between maternal blood-Cr and cord blood-Cr levels ($r=-0.21$, $p<0.05$). Also, another negative correlation was determined between the placental Ni and maternal blood Ni concentrations ($r=-0.27$; $p<0.001$). These findings suggest that the placenta, between the maternal and fetal circulation, can be utilized as a biological indicator for exposure to metals during pregnancy (43).

Study limitation

Our research has some limitations. These include the relatively low number of participants and the small catchment area of the sample population as specimens were only obtained in Ankara, Turkey. In order to define global reference values for these heavy metals in these biological tissues, carefully selected and much larger sample populations would be required. As is demonstrated by the widespread neoteric studies mentioned here, numerous investigators are performing novel and innovative designs in the field of toxicological monitoring of metal levels in human biological materials, including maternal blood, cord blood and placenta. There is thus hope that advances will be forthcoming in the prevention of potential birth defects caused by prenatal exposure to chemicals.

Conclusion

This study showed that quantification and identification of Cr and Ni in biological samples of mother-newborn pairs can be used as evidence of neonatal exposure. The results also reiterate that the placenta is not a perfect preservative against metal ion transport, although the placenta appears as a regulating organ. The measured concentration of Cr and Ni were relatively low compared to other reference ranges and this suggested that exposure to these metals poses little threat negative for the mothers and the newborns in our cohort. Besides, this GFAAS method offers excellent versatility for clinical research laboratories, since the validation was appropriate in terms of ISO 17025 certification. Last but not least, the reported reference values of Cr and Ni in the biological specimens through this paper will provide complementary aid to health professionals in terms of assessment of environmental and occupational exposure.

Ethics Committee Approval: *This scientific work was ethically authorized by the Research Ethics Committee of the Ankara University Faculty of Medicine (approval number: 33-730/July 11, 2011).*

Informed Consent: *Each volunteer provided written informed consent in line with the ethics as recognized in the Declaration of Helsinki (World Medical Association, Declaration of Helsinki, 1964).*

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