

Analysis of the content and correlation of 6 Trace Elements in maternal and fetal blood in Shenyang area.

Yan Qi and Juan Du*

Obstetrics and Gynecology Department, Shengjing Hospital of China Medical University, Shenyang 110004, P. R. China

Abstract

To measure the levels of Zn, Mn, Cu, Pb, Cd, As and other trace elements in bodies of the mother and the fetus and to perform correlation analysis among trace elements. Total of 180 pregnant women hospitalized between October 2008 and Apr 2009 were continuously recruited in this study. Contents of trace element in maternal and umbilical cord blood including Zn, Mn, Cu, Pb, Cd and As etc. were determined using inductively coupled plasma mass spectrometry system. SPSS12.0 software was applied to establish database and conduct statistical analysis. Median concentrations of Zn, Mn, Cu, Pb, As and Cd in maternal blood were respectively 5747.0 µg/L, 29.145 µg/L, 1410.5 µg/L, 32.105 µg/L, 4.435 µg/L, and 1.155 µg/L, and median concentrations of corresponding element in umbilical cord blood were respectively 1793.0 µg/L, 45.489 µg/L, 464.4 µg/L, 21.625 µg/L, 2.976 µg/L, and 0.142 µg/L. Contents of Zn, Cu, Pb, As and Cd in maternal blood were significantly higher than that in umbilical cord blood ($P < 0.05$). Levels of Mn in umbilical cord blood were significantly higher than that in maternal blood ($P < 0.05$). All 6 trace elements in maternal blood had significant positive correlation to corresponding elements in umbilical cord blood ($r: 0.169 \sim 0.378$ and all $P < 0.05$). Multiple linear regression analysis showed that various trace elements in maternal blood were the main determinant of corresponding trace elements in umbilical cord blood. Some trace elements in maternal blood also had certain impact on the levels of other trace elements in umbilical cord blood. This study showed that toxic trace elements Pb, As and Cd existed at higher level in patients from China than developed countries, which is a problem not to be ignored. Trace element levels in neonate were closely related to that in the mother, while levels of trace element in maternal blood had some effects on the levels of other trace element in umbilical cord blood. Therefore, monitoring trace element levels during pregnancy and effective treatment in case of abnormal findings were still important components of eugenics.

Keywords: Trace elements; Heavy metals; Interaction; Umbilical cord blood; Maternal blood

Accepted February 07 2015

Introduction

As modern industry develops, pollution becomes worse and lifestyle is changing. An increasing number of environmental factors are altering our way of lives. In particular, environmental hazards in pregnancy have caused widespread concerns. Trace elements in the human body can be divided into essential trace elements and toxic trace elements. Being common toxic heavy metals in our daily life, Pb, As and Cd etc. had been proved to have strong toxicity on reproductive system and embryo development by animal experiments [1-2], potentially threatening the healthy reproduction of human being. On contrary, Zn, Mn and Cu are essential trace elements in the human body and some studies showed that Zn deficiency was associated with a variety of adverse outcomes of pregnancy [3-4] while too low or too high concentrations of Mn also had adverse effects on

pregnancy [5]. Furthermore, lack of Cu could cause anemia and stunted development [6]. In recent years, some studies tested the concentrations of some essential and toxic trace elements in maternal blood and umbilical cord blood of the neonate [5, 7-10]. However, more systematic tests and analysis on these trace elements in maternal blood and neonatal blood including both essential and toxic trace elements, as well as metal and metalloid elements, were still few. In addition, interaction between nutrient elements and toxic elements has become an emerging area of research in recent years [8, 11], but whether interaction of trace elements in maternal body affects the placental transport of trace elements into the fetus has not been reported. In this paper, we tested the concentrations of 6 trace elements in maternal whole blood and neonatal blood in Shenyang area and analyzed the maternal-fetal correlation of these elements as reported as follows.

Subjects and Methods

Subjects

Total of 180 pregnant women admitted into the Shenjing hospital of China Medical University were selected consecutively from October 2008 to April 2009, aged 21~34 years old, all singleton pregnancies and gestational age of 37-42 weeks. Patients all received 3~10 times of prenatal care and had no major organ diseases, no intake of trace element supplements within the last week, natural vaginal delivery or cesarean section, no neonatal asphyxia or birth defects and with neonatal birth weight range of 2500 ~ 4000 g. All subjects signed informed consent.

Methods

Sample Collection

Maternal whole blood: after signed informed consent, 3ml venous blood was collected from pregnant women at 0 ~ 24h before delivery, ethylenediaminetetraacetic acid (EDTA) was added and mixed to prevent anticoagulation. Umbilical cord blood: after delivery, surface of the umbilical cord was swabbed and disinfected. 3ml cord blood was collected. Sample processing method was the same as the maternal whole blood. Samples were stored at -80°C refrigerator.

Sample preparation

Agilent 7500 CE inductively coupled plasma mass spectrometry system (ICP-MS) (Agilent Inc., United States) was used for determination and analysis of various trace elements in the whole blood. 400 µl of 69% nitric acid was added into 200µl of whole blood sample and Mars-5 microwave digestion oven (CEM Inc., United States) was used for digestion. Digested solution was diluted to about 4.5 ml in pure water and measured on ICP-MS. The material from CORTOX (lot HMB59311, Kaulson Laboratories Inc., United States) was used as quality control. In addition, measurement was repeated for 10% of specimens.

Detected indicators

Essential trace elements Zn, Mn and Cu, as well as toxic trace elements Pb, Cd and As were measured.

Statistical analysis

SPSS 12.0 was used to establish database and perform statistical analysis. Distribution of all elements was

statistically described using median and the lowest to highest quartile. Differences between maternal and umbilical cord blood were detected using Wilcoxon Signed Ranks Test (paired rank test) and Spearman rank correlation analysis was used to analyze the correlation of trace elements levels in maternal and umbilical cord blood. Multiple linear regression (Stepwise) Analysis was applied to analyze the effects of various maternal blood trace elements on umbilical cord blood trace elements. $P < 0.05$ was set as the criteria for statistically significant difference.

Results

Basic information

Total of 180 cases of 21-34 years old pregnant women were included in our study, with average age of (28.4 ± 3.8) years old, average height of (162.1 ± 4.9) cm and body weight of (74.3 ± 8.8) Kg before delivery. Fetus had body weight of (3.455 ± 0.54) Kg and height of (50.7 ± 2.2) cm.

Distribution of trace elements and heavy metal levels in maternal and cord blood

Normal distribution was shown for Cu level in maternal and umbilical cord blood, but non-normal distribution was shown for all other elements. Contents of various elements in maternal and cord blood were listed in Table 1. Detection rates all trace elements in maternal blood and in umbilical cord blood were 100 %.

Correlation analysis of trace elements in maternal and umbilical cord blood

Comparison and correlation analysis results of various elements in maternal and umbilical cord blood were shown in Table 1. Wilcoxon Signed Ranks Test showed that levels of Zn, Cu, Pb, Cd and As in maternal blood were significantly higher than in umbilical cord blood (all $P < 0.05$) while contents of Mn in cord blood were significantly higher than in maternal blood (all $P < 0.001$). Spearman rank correlation analysis showed that levels of all 3 essential and 3 toxic trace elements in maternal and umbilical cord blood had significant positive correlation (correlation coefficient of 0.169 ~ 0.378 and all $P < 0.05$) (Table 1).

Table 1. Content of elements in maternal and umbilical cord blood

| Trace elements | Maternal Blood | | | Umbilical cord blood | | | Z | P* | r | P ^s |
|----------------|----------------|--------------------------------|---------------|----------------------|--------------------------------|----------------|---------|---------|-------|----------------|
| | Median | Q ₁ -Q ₄ | Range | Median | Q ₁ -Q ₄ | Range | | | | |
| Pb(µg/L) | 21.105 | 7.854-35.170 | 0.000-121.70 | 21.625 | 8.846-27.640 | 0.000-436.300 | -1.357 | 0.175 | 0.268 | <0.001* |
| As(µg/L) | 4.435 | 3.551-6.233 | 2.166-30.610 | 2.967 | 2.217-4.428 | 0.000-132.90 | -7.877 | <0.001* | 0.276 | <0.001* |
| Cd(µg/L) | 1.155 | 0.811-1.472 | 0.338-3.119 | 0.142 | 0.021-0.405 | 0.000-16.780 | -11.035 | <0.001* | 0.378 | <0.001* |
| Zn(µg/L) | 5747.0 | 4947.0-6312.0 | 2300.0-8620.0 | 1793.0 | 1526.0-2054.0 | 789.0-13850.0 | -11.285 | <0.001* | 0.107 | 0.153 |
| Cu(µg/L) | 1410.5 | 1251.5-1546.8 | 539.9-2636.0 | 464.4 | 405.9-531.1 | 193.5-1967.0 | -11.548 | <0.001* | 0.077 | 0.301 |
| Mn(µg/L) | 29.145 | 24.525-34.775 | 14.410-70.230 | 45.895 | 38.748-56.873 | 17.080-158.500 | -10.079 | <0.001* | 0.072 | 0.336 |

Note: Q₁-Q₄: lowest quartile to highest quartile; Range: minimum to maximum. * Wilcoxon Signed Ranks Test (maternal blood vs. cord blood) ^s Spearman rank correlation (maternal blood vs. cord blood)

Table 2. Effects of various trace elements in umbilical blood

| Trace elements in cord blood | Trace elements in umbilical blood affecting elements in cord blood | Unstandardized coefficients | | Standardized | | |
|------------------------------|--|-----------------------------|---------|--------------|--------|---------|
| | | β | SE | B | t | P |
| Pb | Cd | 24.320 | 1.404 | 0.954 | 17.318 | <0.001* |
| As | Cd | 7.469 | 1.075 | 0.972 | 6.950 | <0.001* |
| | Zn | -2.015 | 1.169 | -0.208 | -1.723 | 0.087 |
| Cd | Pb | 0.013 | 0.002 | 0.332 | 8.685 | <0.001* |
| | As | 0.027 | 0.004 | 0.211 | 6.847 | <0.001* |
| | Cu | -0.001 | 0.001 | -0.091 | -3.754 | <0.001 |
| Zn | Cu | 0.001 | 0.000 | 0.169 | 5.572 | <0.001* |
| | Mn | 0.004 | 0.002 | 0.058 | 1.675 | 0.096 |
| | As | -0.009 | 0.005 | -0.083 | -1.786 | 0.076 |
| Cu | Zn | 153.509 | 27.549 | 0.890 | 5.572 | <0.001* |
| | Mn | 2.391 | 0.839 | 0.223 | 2.848 | 0.005* |
| | Cd | -125.722 | -23.835 | -0.918 | -5.275 | <0.001* |
| Mn | Zn | 2.807 | 1.380 | 0.175 | 2.034 | 0.043* |
| | Cu | 0.020 | 0.006 | 0.218 | 3.400 | 0.001* |

Multivariate analysis of trace elements in umbilical cord blood:

As shown in Table 2, all trace elements in maternal blood were the main determinants of the corresponding trace elements in umbilical cord blood, i.e., there was a significant positive correlation between the maternal element and fetal element. In addition, multiple linear regression analysis showed that maternal Zn level could independently reduce the concentration of Cd and As in umbilical cord blood. Maternal Pb level was negatively correlated with the level of Cu in umbilical cord blood. Furthermore, maternal As concentration was negatively correlated with the Mn level in umbilical cord blood.

Discussion

This project studied the general population in Shenyang area to conduct a systematic investigation on levels of essential trace elements Zn, Mn and Cu, as well as toxic trace elements Pb, Cd and As in pregnant women and fetuses. In addition, correlation between trace elements in the mother and the fetus was analyzed and the interaction among trace elements was explored.

As an essential trace element in the human body, Zn plays important roles in embryonic development of the fetus. In this study, median Zn level in maternal whole blood was 5747.0 $\mu\text{g/L}$ in Shenyang area, slightly lower than that in Shanghai area (6312.5 $\mu\text{g/L}$) [5], but significantly higher than the level reported in Japan (5561 $\mu\text{g/L}$) [11]. Meanwhile, umbilical cord blood Zn level (1793 $\mu\text{g/L}$) in this study was also lower than that of Shanghai area (2250 $\mu\text{g/L}$) while higher than that of Japanese population (1955 $\mu\text{g/L}$) [11], suggesting that blood Zn level in different regions was also different. As another essential trace element in the body, Mn is transported between fetus and the mother through active transport process [12]. This

study showed that Mn content in the fetal body (45.895 $\mu\text{g/L}$) was significantly higher than the mother (29.145 $\mu\text{g/L}$), while average Mn levels in umbilical cord blood was higher than that reported in Japan (umbilical cord blood: 28 $\mu\text{g/L}$) and the United States (umbilical cord blood: 42 $\mu\text{g/L}$) [12], while the average Mn levels in maternal blood was lower than that reported in Japan (maternal: 31 $\mu\text{g/L}$) and the United States (maternal: 35 $\mu\text{g/L}$) [12]. Cu is also an essential trace element in the human body. Deficiency in Cu could cause anemia and stunted growth etc. In this study, Cu level in umbilical cord blood (464.4 $\mu\text{g/L}$) was higher than that in Canadian area (408 $\mu\text{g/L}$), while Cu level in maternal blood (1410.5 $\mu\text{g/L}$) was significantly lower than average level in Arctic Canadian area (2160 $\mu\text{g/L}$) [13].

In this study, median Pb level in maternal blood was 32.105 $\mu\text{g/L}$, lower than the maternal blood Pb level in late pregnancy in Shanghai area (64.32 $\mu\text{g/L}$) [5] while median Pb level in umbilical cord blood was 21.625 $\mu\text{g/L}$, also significantly lower than that of Shanghai in 2001 (73 $\mu\text{g/L}$) [14]. The reason may be that Shenyang has fewer industries directly involved Pb. However, Pb level in Shenyang area was still relatively higher than that reported in the United States in 2005 (mean maternal blood Pb level of 19.3 $\mu\text{g/L}$ in late pregnancy) [15]. Animal studies suggested that As and Cd had embryo toxicity and teratogenicity [2,16] while population data also showed that As and Cd exposure during pregnancy might cause premature birth, stillbirth and low birth weight [17, 18]. Median maternal blood Cd level in this study was 1.155 $\mu\text{g/L}$, significantly higher than that reported in Shanghai China (0.84 $\mu\text{g/L}$) [5], the United States (0.3 $\mu\text{g/L}$) and Russia (0.29 $\mu\text{g/L}$) [8, 19], but lower than that in Taiwan China (1.30 $\mu\text{g/L}$) [20]. Umbilical cord blood Cd level (0.142) was also significantly lower than that reported in Shanghai China

(0.32 µg/L) and Taiwan (0.78 µg/L). In addition, median As level in maternal blood was 4.435 µg/L, higher than reported in Sweden (1.0-2.4 µg/L) but lower than in Taiwan, China (6.8 µg/L). Furthermore, As level in umbilical cord blood (2.967 µg/L) was higher than that reported in Shanghai China (2.84 µg/L) [5] but lower than that in Taiwan China (7.9 µg/L) [20]. Overall, levels of these 6 elements were within the normal reference range in majority of subjects. However, distribution of different element concentration had apparent individual variation, i.e., the maximal value and the minimal value could differ by hundreds or even thousands of folds, suggesting that individual placenta had different barrier effects on trace elements due to significant individual variation.

Necessary nutrients for fetal growth and development were all obtained from the mother through placenta. Therefore, to explore the content and difference of whole blood trace elements in maternal and fetal body is of great significance for the in-depth study of fetal development in uterus and its impact factors, thereby to explore the correlation between trace elements in the body and human health. In this study, correlation analysis on distribution of essential trace elements in maternal blood and umbilical cord blood suggested that levels of essential trace elements Zn and Cu had significant positive correlation between maternal and umbilical cord blood, suggesting the dependence of fetus on the mother to obtain these elements. Lack of Zn was related to many adverse pregnancy outcomes [3,4]. Cu involves in growth and metabolism of cardiovascular system and promotes synthesis of nucleic acid and protein, while Mn affects glucose metabolism by affecting the metabolism of insulin [21]. Therefore, pregnant women with deficiencies in the above elements should supplement these elements in a timely manner. Levels of Mn in maternal blood were significantly higher than the cord blood, indicating that Mn elements were actively transported from the maternal body to the fetus, so in case of relatively low concentration of these elements in maternal body, supply of the fetus could still be prioritized through active transport through the placenta.

In this study, correlation analysis on distribution of toxic trace elements in maternal and umbilical cord blood suggested that toxic trace elements Pb, Cd and As in the maternal blood had positive correlation with that in umbilical cord blood and maternal blood Pb, Cd and As levels were significantly higher than in umbilical cord blood, indicating that these 3 elements could enter fetal body through placenta barrier following concentration gradient. However, since transport of these elements from the mother to the fetus was affected by placenta barrier at a certain degree, it is not a simple diffusion process

In recent years, interaction among various trace elements in the body has become a research focus. In this study, *Biomed Res- India 2015 Volume 26 Issue 3*

multiple linear regression analysis showed that trace element levels in pregnant women were a primary factor to affect the level of corresponding trace element in the fetus. In addition, maternal blood Zn level had significant independent effect on the levels of Cd and As in umbilical cord blood after multi-factored adjustment, which significantly reduced the levels of Cd and As in the fetus. This may be due to the similar chemical properties of Zn and Cd, so there is likely an antagonistic effect between these two. However, we do not understand the exact mechanism of how Zn affects the placental transport of Cd and As into the fetus [22]. In addition, our study also found that maternal blood Pb level had independent negative correlation to umbilical cord blood Mn level while the levels of As in maternal blood had significant negative correlation to umbilical cord blood Mn level. These data should help to clarify the mechanism of how maternal blood trace elements affect placental transport of other trace elements in the future.

In summary, this study comprehensively analyzed the distribution status of essential trace elements Zn, Mn and Cu and toxic trace elements Pb, As and Cd in pregnant women in Shenyang area. In addition, we also confirmed that trace element concentrations in neonate and the mother were closely related while levels of trace element in maternal blood had certain impact on the level of other trace elements in umbilical cord blood. Therefore, monitoring of trace elements during pregnancy and effective responsive measures are still an important component of eugenics.

References

1. Bellés M, Albina ML, Sánchez DJ, Corbella J, Domingo JL. Interactions in developmental toxicology: effects of concurrent exposure to lead, organic mercury, and arsenic in pregnant mice. *Arch Environ Contam Toxicol* 2002; 42: 93-98.
2. Srivastava S, Mehrotra PK, Srivastava SP, Siddiqui MK. Some essential elements in maternal and cord blood in relation to birth weight and gestational age of the baby. *Biol Trace Elem Res* 2002; 86: 97-105.
3. Danesh A, Janghorbani M, Mohammadi B. Effects of zinc supplementation during pregnancy on pregnancy outcome in women with history of preterm delivery: A double-blind randomized, placebo-controlled trial. *J Matern Fetal Neonatal Med* 2009; 29: 1-7.
4. Watson PE, McDonald BW. Seasonal variation of nutrient intake in pregnancy: effects on infant measures and possible influence on diseases related to season of birth. *Eur J Clin Nutr* 2007; 61: 1271-1280.
5. Wang P, Tian Y, Shi Y, Zou XY, Gao Y, Wang MM, Gong YL, Zhang YB, Yan CZ. Study on maternal-fetal status of Pb, As, Cd, Mn and Zn elements and the Influence factors. *Chinese Journal of Preventive Medicine* 2008; 42: 722-726.
6. Roberfroid D, Huybregts L, Lanou H, Henry MC, Meda N, Menten J, Kolsteren P. MISAME Study

- Group. Effects of maternal multiple micronutrient supplementation on fetal growth: a double-blind randomized controlled trial in rural Burkina Faso. *Am J Clin Nutr* 2008; 88: 1330-1340.
7. Gu GX, Xu PJ, Li L. Blood Cadmium Levels of Mother and Neonate and Its Relevant Influential Factors. *Industrial Health and Occupational Diseases* 2007; 03: 150-153.
 8. Butler Walker J, Houseman J, Seddon L, McMullen E, Tofflemire K, Mills C, Corriveau A, Weber JP, LeBlanc A, Walker M, Donaldson SG, Van Oostdam J. Maternal and umbilical cord blood levels of mercury, lead, cadmium, and essential trace elements in Arctic Canada. *Environ Res* 2006; 100: 295-318.
 9. Cai DB, Hong JM, Huang RQ. Study on whole blood trace elements in 170 pairs of Neonates and Mothers. *GuangDong Trace Elements Science* 1995; 2: 55-59.
 10. Osman K, Akesson A, Berglund M, Bremme K, Schütz A, Ask K, Vahter M. Toxic and essential elements in placentas of Swedish women. *J Environ Monit* 2001; 3: 177-184.
 11. Vigh M, Yokoyama K, Ramezanzadeh F, Dahaghin M, Sakai T, Morita Y, Kitamura F, Sato H, Kobayashi Y. Lead and other trace metals in preeclampsia: a case-control study in Tehran, Iran. *Environ Res* 2006; 100: 268-275.
 12. Takser L, Lafond J, Bouchard M, St-Amour G, Mergler D. Manganese levels during pregnancy and at birth: relation to environmental factors and smoking in a Southwest Quebec population. *Environ Res* 2004; 95: 119-125.
 13. Jody Butler Walker, Jan Houseman Laura Seddon, Ed McMullen. Maternal and umbilical cord blood levels of mercury, lead, cadmium, and essential trace elements in Arctic Canada. *Environmental Research* 2006; 100: 295-318.
 14. Tang GJ, Gu P, Ding LD, Cai MJ, He JM, Ao LM. Effects of low level lead exposure on fetus during pregnancy. *The Journal of Clinical Pediatrics* 2001; 19: 242-245.
 15. Harville EW, Hertz-Picciotto I, Schramm M, Watt-Morse M, Chantala K, Osterloh J, Parsons PJ, Rogan W. Factors influencing the difference between maternal and cord blood lead. *Occup Environ Med* 2005; 62: 263-269.
 16. Rodríguez VM, Carrizales L, Mendoza MS, Fajardo OR, Giordano M. Effects of sodium arsenite exposure on development and behavior in the rat. *Neurotoxicol Teratol* 2002; 24: 743-750.
 17. Nishijo M, Nakagawa H, Honda R, Tanebe K, Saito S, Teranishi H, Tawara K. Effects of maternal exposure to cadmium on pregnancy outcome and breast milk. *Occup Environ Med* 2002; 59: 394-396, 397.
 18. Milton AH, Smith W, Rahman B, Hasan Z, Kulsum U, Dear K, Rakibuddin M, Ali A. Chronic arsenic exposure and adverse pregnancy outcomes in bangladesh. *Epidemiology* 2005; 16: 82-86.
 19. Zhang YL, Zhao YC, Wang JX, Zhu HD, Liu QF, Fan YG, Wang NF, Zhao JH, Liu HS, Ou-Yang L, Liu AP, Fan TQ. Effect of environmental exposure to cadmium on pregnancy outcome and fetal growth: a study on healthy pregnant women in China. *J Environ Sci Health A Tox Hazard Subst Environ Eng* 2004; 39: 2507-2515.
 20. Soong YK, Huang HY, Huang CC, Chu NS. Successful pregnancy after D-penicillamine therapy in a patient with Wilson's disease. *J Formos Med Assoc* 1991; 90: 693-696.
 21. Al-Saleh E, Nandakumaran M, Al-Shammari M, Al-Falah F, Al-Harouny A. Assessment of maternal-fetal status of some essential trace elements in pregnant women in late gestation: relationship with birth weight and placental weight. *J Matern Fetal Neonatal Med* 2004; 16: 9-14.
 22. Zhang YL, Zhao YC, Fan YG, Liu QF, Wang NF, Liu AP. Effect of Essential elements Zinc, Copper, and Selenium on Placental Cadmium Transport. *Acta Nutrimenta Sinca* 2004; 26: 450-453.

Correspondence to:

Juan Du
Obstetrics and Gynecology Department
Shengjing Hospital of the China Medical University
Shenyang 110004, P. R. China;

•