Lead and other trace metals in preeclampsia: A case–control study in Tehran, Iran

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Abstract

To assess the effects of environmental exposures to trace metals on the incidence of preeclampsia, concentrations of lead (Pb), antimony (Sb), manganese (Mn), mercury, cadmium, cobalt and zinc in umbilical cord blood (UCB) and mother whole blood (MWB) were measured in 396 postpartum women without occupational exposure to metals in Tehran, Iran, using inductively coupled plasma mass spectrometry. Mother’s ages ranged from 15 to 49 (mean 27) years. Preeclampsia was diagnosed in 31 subjects (7.8%). Levels of Pb, Sb and Mn in UCB were significantly higher in preeclampsia cases [mean ± SD of 4.30 ± 2.49 µg/dl, 4.16 ± 2.73 and 46.87 ± 15.03 µg/l, respectively] than in controls [3.52 ± 2.09 µg/dl, 3.17 ± 2.68 and 40.32 ± 15.19 µg/l, respectively] (P < 0.05). The logistic regression analysis revealed that one unit increase in the common logarithms of UCB concentration of Pb, Sb or Mn led to increase in the risk of preeclampsia several-fold; unit risks (95% CI) were 12.96 (1.57–107.03), 6.11 (1.11–33.53) and 34.2 (1.81–648.04) for Pb, Sb and Mn, respectively (P < 0.05). These findings suggest that environmental exposure to Pb, Sb and Mn may increase the risk of preeclampsia in women without occupational exposure; levels of metals in UCB to be sensitive indicators of female reproductive toxicity as compared with those in mother MWB. Further studies are necessary to confirm these findings, especially on Sb and Mn.

Keywords: Preeclampsia; Umbilical cord blood; Lead; Antimony; Manganese

1. Introduction

Some metals are essential to maintain the metabolism of human body, whereas they can lead to poisoning because they tend to bioaccumulate (Rani, 2000). The Industrial Revolution, which increased opportunities of occupational and environmental exposure to metals among women, revealed their adverse effects on pregnancy. Recent researches have begun to focus on the health effects of exposure to low-level of metals because they may be toxic at the levels previously thought to have no adverse effect on human (Järup, 2003). Furthermore, during pregnancy, blood levels of some metals increase because of increase in bone turnover (Hertz-Piccitto et al., 2000; Silbergeld, 1991) and increased gastrointestinal absorption (Leazer et al., 2002) or other unknown mechanisms.

Effects of lead (Pb) on reproductive system have been studied intensively, e.g. pregnancy outcome (Andrews et al., 1994) and pregnancy hypertension (Rabinowitz et al., 1987; Rothenberg et al., 1999; Vigeh et al., 2004),...
yielding controversial results. Only few reports have been conducted on the effects of complexes of metals on pregnancy (Abdelrahman and Kincaid, 1993; Osada et al., 2002); adequate descriptions of metals in various complications of pregnancy are lacking.

Preeclampsia, pregnancy-specific syndrome characterized by hypertension and proteinuria, is a multisystem disorder and an important cause of morbidity for both mother and fetus, but its etiology remains unknown. Environmental factors may have a role in this disease occurrence (Cunningham et al., 2001). A seasonal variability in the occurrence of preeclampsia with a peak in winter months and a minimum in the summer; suggest environmental factor effects on this disease (Magnus and Eskild, 2001). Dawson et al. (1999) demonstrated significant increase in Pb, cadmium (Cd), copper (Cu) and magnesium (Mg) and decrease in zinc (Zn) in amniotic fluid of preeclamptic cases. Decrease of Zn and increase of Cu have been also observed in placenta of preeclampsia cases (Angell and Lavery, 1982).

The study presented here is aimed at evaluating the relationship between trace metals concentrations in umbilical cord blood (UCB) and mother whole blood (MWB) with preeclampsia among those women without occupational exposure to trace metals in Tehran, Iran. The study is first focused on Pb as this metal has well-known adverse effects on renal system and blood pressures (Batuman, 1993; Batuman et al., 1983; Gonick and Behari, 2002; Landrigan et al., 1995; Vupputuri et al., 2003; Wedeen et al., 1986; Wedeen, 1991) and suggested to be a risk of pregnancy hypertension (Vigeh et al., 2004). The study is also exploratory, as we measure UCB and MWB levels for other metals of which effects on blood pressure and/or renal system have been suggested by some human and animal studies, viz. antimony (Sb) (ATSDR, 1995; Brieger et al., 1954; Cotten and Logan, 1966; Hepburn et al., 1994; Rai et al., 1994; Rossi et al., 1987; Veiga et al., 1983), manganese (Mn) (Bencko and Cikrt, 1984; Jynge et al., 1997; Khapre and Rajapurkar, 1969; Kimura et al., 1978; Saric and Hrustic, 1975; Shutte et al., 2003; Wolf and Baum, 1983), mercury (Hg) (Koyun et al., 2002) and Cd (Wedeen, 1991; Kosanovic et al., 2002; Varoni et al., 2003).

2. Subjects and methods

2.1. Subjects

The study was conducted as a joint research of the University of Tokyo, Mie University and Tehran University of Medical Sciences, from April 2003 to January 2004 in two teaching hospitals in Tehran, Iran. The ethical committees at the Vali-e-Asr Reproductive Health Research Center, Faculty of Medicine, University of Tehran Medical Sciences, Tehran, Iran, approved the research protocol and the survey was conducted under their supervision. All subjects were informed of the purpose and procedure of the study verbally and their participation to the study was on a purely voluntary basis.

Eighty-five percent of women who delivered at the two hospitals during the study period agreed to participate into the study, i.e. 433 postpartum volunteers were surveyed. None of them had chronic conditions such as heart disease, diabetes, cancer and renal failure. Among subjects, 35 women positive for the hepatitis B virus antigen and 2 women positive for the HIV were not accepted. Thus, 396 subjects were included into the study.

The criterion of preeclampsia in the survey were (1) systolic blood pressure $\geq 140$ mm Hg and/or diastolic blood pressure $\geq 90$ mm Hg after 20th week of gestation and (2) proteinuria $\geq 300$ mg per 24 h or persistent $\geq 1$+ proteinuria by dipstick in urine samples.

2.2. Collection and analysis of blood samples

At the time of delivery, UCB samples were collected using heparinized metal-free vacuum tubes. MWB samples were collected in post partum ward within 24 h after the delivery. The blood samples were stored at $-20^\circ$C until analysis.

Blood samples (0.1 ml) were diluted 10-fold with pure water to lyse blood cells and then mixed with 4 ml of reagent containing ethylenediaminetetraacetic acid (0.5 g/l), Triton $\times 100$ (0.5 g/l) and ammonia (5 ml/l), and 100 ml of internal standard solution containing 50 ng of bismuth, cerium and yttrium. Concentrations of metals (Pb, Sb, Mn, Hg, Cd, Co and Zn) in the samples were measured using an inductively coupled plasma mass spectrometry (Model ICP-MS, HP 4500, Hewlett-Packard, USA). All measurements were conducted in duplicate; if the result was dubious, repeat measurement was made. For instruments standard checking, throughout measurement, at least 15% of the analyses were controls, and 10% were blanks. Coefficient of variation for measurement was less than 5% for Pb, 12% for Cd and 10% for other metals. Participation in the inter-comparison program conducted by German Society of Occupational Medicine and Environmental Medicine ensured external quality control (The German Quality Assessment Scheme, Website; Schaller et al., 2000). Because of technical reasons such as loss of samples and abnormal results for the internal standards, some data were missing (Table 1).
2.3. Collection of demographic variables

Just after the collection of MWB samples, subjects were interviewed using structured questionnaires to obtain socioeconomic background, anthropometric variables and other characteristics such as medical and reproductive history, habits, and probability of metal exposure. Pregnancy weight gain just before the delivery, compared with pre-gravid one, was calculated. Body mass index (BMI) just before the delivery was obtained as the weight (kg) divided by the square of height (m²).

2.4. Statistical analysis

Wilcoxon rank sum test, \( \chi^2 \)-test and Fisher exact probability test were employed to compare the subject’s characteristics and trace metals concentrations between preeclampsia and non-preeclampsia subjects. Spearman’s rank correlation coefficients were calculated to assess associations between blood pressures and trace metal concentrations. In the logistic regression analysis (stepwise method), preeclampsia (\( y = 1 \)) or not (\( y = 0 \)) was defined as dependent variable; independent variables were BMI, nulliparity, education, multiple gestations (twin or triple), pregnancy weight gain, age, hematocrit, and the log of UCB and MWB concentrations of trace metals. SPSS version 10.1 was employed for the analysis.

3. Results

Thirty-one subjects (7.8%) were diagnosed as preeclampsia. In the preeclampsia cases, concentrations of Pb, Sb and Mn in UCB were significantly higher than those in controls (Table 1). Similarly, BMI was significantly higher and nulliparity and multiple gestations were more frequent than controls (Table 1). Ratios of UCB to MWB concentrations of Pb and Mn in preeclampsia were significantly higher than those in controls (Table 2). Concentrations of Sb in MWB, Pb

### Table 1
Comparison of demographic/clinical characteristics between 31 preeclamptic and 365 non-preeclamptic subjects

<table>
<thead>
<tr>
<th></th>
<th>Preeclampsia Mean (SD, range)</th>
<th>Non-preeclampsia Mean (SD, range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>26.0 (4.0, 19–35)</td>
<td>26.9 (5.7, 15–49)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.59 (0.04, 1.50–1.67)</td>
<td>1.60 (0.05, 1.41–1.75)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.39 (12.12, 57.0–110.0)</td>
<td>75.45 (13.21, 46.0–120.0)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>31.52 (5.27, 23.1–47.0)*</td>
<td>29.44 (4.81, 23.1–47.0)*</td>
</tr>
<tr>
<td>Pregnancy weight gain (kg)</td>
<td>14.58 (4.97, 3.0–30.0)</td>
<td>12.95 (4.30, 3.0–30.0)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>37.41 (4.67, 24.9–46.6)</td>
<td>38.25 (6.43, 22.6–47.5)</td>
</tr>
</tbody>
</table>

**Mother whole blood**

<table>
<thead>
<tr>
<th></th>
<th>Preeclampsia</th>
<th>Non-preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb (μg/dl)</td>
<td>5.09 (2.01, 1.6–10.1)</td>
<td>4.82 (2.22, 1.7–24.6)</td>
</tr>
<tr>
<td>Sb (μg/l)</td>
<td>3.69 (2.83, 1.6–16.7)</td>
<td>3.19 (2.18, 0.8–21.0)</td>
</tr>
<tr>
<td>Mn (μg/l)</td>
<td>17.05 (6.81, 7.2–36.9)</td>
<td>18.52 (5.75, 6.9–39.4)</td>
</tr>
<tr>
<td>Hg (μg/l)</td>
<td>1.35 (0.74, 0.0–3.4)</td>
<td>1.34 (1.19, 0.0–13.2)</td>
</tr>
<tr>
<td>Cd (μg/l)</td>
<td>0.54 (0.31, 0.0–1.30)</td>
<td>0.50 (0.30, 0.0–6.30)</td>
</tr>
<tr>
<td>Co (μg/l)</td>
<td>0.50 (0.21, 0.2–1.1)</td>
<td>0.54 (0.16, 0.1–2.6)</td>
</tr>
<tr>
<td>Zn (μg/l)</td>
<td>5200 (1444, 2700–8476)</td>
<td>5561 (1057, 1587–8451)</td>
</tr>
</tbody>
</table>

**Umbilical cord blood**

<table>
<thead>
<tr>
<th></th>
<th>Preeclampsia</th>
<th>Non-preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb (μg/dl)</td>
<td>4.30 (2.49, 1.8–14.6)*</td>
<td>3.52 (2.09, 0.8–27.0)*</td>
</tr>
<tr>
<td>Sb (μg/l)</td>
<td>4.16 (2.73, 1.1–14.0)**</td>
<td>3.17 (2.68, 1.0–26.0)**</td>
</tr>
<tr>
<td>Mn (μg/l)</td>
<td>46.87 (15.03, 24.1–85.0)*</td>
<td>40.32 (15.19, 13.0–118.2)*</td>
</tr>
<tr>
<td>Hg (μg/l)</td>
<td>1.69 (1.19, 0.0–5.10)</td>
<td>1.70 (1.33, 0.0–13.1)</td>
</tr>
<tr>
<td>Cd (μg/l)</td>
<td>0.34 (0.39, 0.0–1.70)</td>
<td>0.35 (0.44, 0.0–6.3)</td>
</tr>
<tr>
<td>Co (μg/l)</td>
<td>0.52 (0.22, 0.30–1.30)</td>
<td>0.48 (0.21, 0.1–2.6)</td>
</tr>
<tr>
<td>Zn (μg/l)</td>
<td>1819 (473, 1343–3663)</td>
<td>1955 (632, 1062–6572)</td>
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</tbody>
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<table>
<thead>
<tr>
<th></th>
<th>No. of yes (%)/no (%)</th>
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<tbody>
<tr>
<td>Nulliparous</td>
<td>19 (58)/12 (42)*</td>
</tr>
<tr>
<td>Multiple gestation</td>
<td>3 (10)/28 (90)*</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>0 (0)/31 (100)</td>
</tr>
<tr>
<td>Alcohol drinking</td>
<td>0 (0)/31 (100)</td>
</tr>
<tr>
<td>Education (≥High school)</td>
<td>16 (51)/15 (49)</td>
</tr>
</tbody>
</table>

\*P<0.05 and **P<0.01, between preeclampsia and non-preeclampsia subjects.

Number of missing values = a2, b3, c1, d17, e27, f19, g5, h4.

1Wilcoxon rank sum test.

\( \chi^2 \)-test, Fisher’s exact test when appropriate.
in UCB, and UCB/MWB ratios of Pb and Mn were significantly correlated with blood pressures in all subjects combined (Table 3). The logistic regression analysis showed that a combination of UCB levels of Pb, Sb, Mn and BMI significantly predicted of the occurrence of preeclampsia (Table 4).

4. Discussion

Levels of Pb, Sb and Mn in UCB were significantly higher in preeclampsia cases than in non-preeclampsia; reflecting these increases, UCB/MWB ratios of Pb and Mn were significantly higher in preeclampsia cases. The logistic regression analysis showed that the increase in UCB levels of these metals led to elevation of the risk of preeclampsia. Furthermore, Sb in MWB, Pb in UCB, and UCB/MWB ratios of Pb and Mn were significantly correlated with blood pressures in all subjects combined, though such correlations were not observed in preeclampsia cases probably due to small number of patients. Thus, the present study suggests potential roles of Pb, Sb and Mn as risk factors of preeclampsia among women without occupational exposure.

Dawson et al. (1999) showed that the levels of Pb, in amniotic fluid elevated in preeclamptic subjects at the third trimester of pregnancy. Our previous study demonstrated that maternal blood Pb was increased in gestational hypertension (with or without proteinuria) (Vigeh et al., 2004). Rabinowitz et al. (1987) found that maternal blood levels of Pb (mean = 6.9 μg/dl) were associated with pregnancy hypertension at the time of delivery. These studies, together with the findings in the present study, indicate that Pb could cause preeclampsia. As it has been shown that lead affects blood pressure and/or renal system (Batuman, 1993; Batuman et al., 1983; Gonick and Behari, 2002; Landrigan et al., 1995; Vupputuri et al., 2003; Wedeen et al., 1986; Wedeen, 1991), such adverse effects of this metal may be enhanced during pregnancy. Angell and Lavery (1982) failed to demonstrate significant relationship between UCB levels of Pb and preeclampsia, however. The difference might have been due to their subjects are younger (mean of 21.1 year); studies are necessary to elucidate the reason further.
Evidence has been given that Pb increases thromboxane in kidney (Fels et al., 1998) and noradrenaline and adrenaline in plasma (Carmignani et al., 2000) as well as enhances the sensitivity to angiotensin II in mesenteric vessels (Skoczylas and Behari, 1999). Pb is also reported to reduce plasma nitric oxide (NO), which is a known vasodilator (Carmignani et al., 2000). Pb might increase the risk of preeclampsia through the influence on these biochemical mediators, as enhanced formation of endothelin and thromboxane, increased vascular sensitivity to angiotensin II, thereby decreased formation of NO were underlying the vasoconstriction in preeclampsia (Granger et al., 2001). In a hypothesis raised by Gonick and Behari (2002), increase in reactive oxygen species is essential in the changes in biochemical mediators caused by Pb.

Mean levels of Pb in MWB in the present study were lower than that in the previous study (5.26 μg/dl) (Vigeh et al., 2004), as Tehran laws had prohibited the use of leaded gasoline since 2002. However, the effects of Pb on fetus should be surveyed in a further study, because the placenta is not noticeable barrier to Pb in the present study that the levels of Pb in UCB reached 73% of those in MWB.

ATSDR (1995) reported that increased blood pressure is a cardiovascular effect of Sb. An experimental study (Rossi et al., 1987) showed that Sb decreased hypotensive response to acetylcholine in rats given drinking water containing 1 mg/dl of antimony trichloride for 60 days, though those given 0.1 or 1 mg/dl of this compound did not reveal hypertension. Brieger et al. (1954) observed 113 workers exposed to 0.58–5.5 mg/m³ of antimony trisulfide for 8 months to 2 years, and found 14 cases of hypertension (>150/90 mm Hg of blood pressure). In the present study, Sb increased in UCB of preeclamptic subjects and was significantly correlated with systolic blood pressures for MWB. Also, renal dysfunction has been demonstrated in Leishmaniasis patients received sodium stibogluconate or meglumine antimonate (containing pentavalent Sb) of 10–20 mg/kg/day for more than month (Veiga et al., 1983; Rai et al., 1994). Thus Sb might have led to preeclampsia, affecting blood pressures and/or renal system. As the levels of Sb in the present study, in both UCB and MWB, were several-fold higher than observed by Krachler et al. (1999), such levels of Sb could be a risk factor for preeclampsia. By contrast, it has been observed that repeated daily injection of sodium antimony dimercaptosuccinate (10.0 mg/kg) for 2–4 days decreased diastolic blood pressures in dogs (Cotten and Logan, 1966) and that 12 men received 20 mg/kg/day of sodium stibogluconate (containing pentavalent Sb) for 20 days had a decrease in systolic and diastolic blood pressures. Effects of Sb on blood pressures should be investigated further by an epidemiological study.

In the present study, UCB levels of Mn were higher in preeclamptic subjects, and UCB/MWB ratio of Mn was correlated with systolic blood pressure. This may agree with the observations of higher plasma activity of renin in those subjects exposed to Mn (Niu et al., 2001) and enhancements of the increase in blood pressure by adrenaline, nor-adrenaline and isopropyl-noradrenaline in dogs given Mn (Khapre and Rajapurkar, 1969). By contrast, Saric and Hrustic (1975) reported that ferroalloy plant workers exposed to 0.39–20.44 mg/m³ of Mn at the workplace showed significantly lower systolic and diastolic blood pressures than workers with exposure levels less than 0.30 mg/m³. Experimental animal studies demonstrated a decrease in blood pressure by intravenous infusion of Mn salt solution (Bencko and Cikrt, 1984; Jynge et al., 1997; Kimura et al., 1978; Wolf and Baum, 1983). As it has been reported that dietary Mn is positively related to pulse pressure in hypertensive but not in normotensive children, effects of Mn on blood pressure should be examined further by an epidemiological study among general population.

Mn levels in UCB were more than twice in MWB in the present study. Similarly, it has been reported that among healthy females, Mn levels in UCB were higher than in MWB (Takser et al., 2004; Audrey et al., 2002). This might be the result of an active placental transfer (Krachler et al., 1999). Also, the MWB level of Mn in the present study and other studies (Spencer and Dietz, 1999; Takser et al., 2004) were several times higher than in non-pregnant women in reproductive age (20–40 years) (Baldwin et al., 1999). Effects of Mn on fetus also should be investigated further.

Despite the above all arguments, the observations of the present study that UCB levels of Pb, Sb and Mn showed relationships to preeclampsia more clearly than MWB levels of these metals did may raise a question about the underlying mechanisms. It may be hypothesized that increase in exposure to these metals had caused maternal hypertension and proteinuria, but elevations in MWB had been soon dissembled possibly by transfer to UCB and/or feto-placenta systems. Alternatively, biological changes in feto-placenta systems might have been essential in metal-related preeclampsia, although both maternal and feto-placental factors are suspected as pathogenesis of preeclampsia (Cross, 2003).

Decreased concentrations of Zn in placenta and amniotic fluid were observed in preeclampsia cases (Brophy et al., 1985; Dawson et al., 1999). Blood pressure (Koyun et al., 2004) and pregnancy hypertension (Kosanovic et al., 2002) were reported to be related to increased blood concentrations of Hg and Cd, respectively. An experimental study showed increased blood pressure in Cd exposed animals (Varoni et al., 2003). Brophy et al. (1985) reported slight differences between concentrations of Co in preeclamptic subjects...
and healthy women. However, we failed to demonstrate significant differences in concentrations of Zn, Hg, Cd and Co between preeclamptic subjects and healthy postpartum women either for MWB or UCB. Reasons for discrepancies should be investigated further.

BMI of preeclamptic women were higher than that of the controls and remained a significant risk factor of preeclampsia in the logistic regression analysis. Wolf et al. (2001) reported higher BMI in preeclampsia (mean 29 kg/m²) than in normal gestation subjects (mean 23 kg/m²). Hrazdilová et al. (2001) reported that increase in BMI increase the incidence of preeclampsia. Thus, the result of the present study coincides with previous observations of increase in the risk of preeclampsia among subjects with higher BMI.

The present study showed preeclampsia was more frequent in women with multiple gestations and nulliparous. Sibai et al. (2000) reported a significant increase of the incidence of preeclampsia in women with twin gestations compared with singletons (13% versus 5%). Coonrod et al. (1995) observed increase in the risk of preeclampsia in twin pregnancy and nulliparity (Odds ratio = 3.5 and 4.0, respectively). Thus, multiple gestations and nulliparity could be risk factors for preeclampsia.

A dose–response relationship between maternal tobacco smoking and alcohol consumption with UCB levels of Pb in newborns has been observed (Rhayinds and Levallois, 1997). Alcohol drinking and cigarette smoking also raise the risk of preeclampsia (Mostello et al., 2002). By contrast, smoking and drinking showed no effects on preeclampsia or Pb levels in the present study. This might be attributed to very low percentage of smokers and alcohol consumers in the study subjects.

In summary, concentrations of Pb, and possibly Sb and Mn, in UCB might be sensitive indicators of preeclampsia among women without occupational exposure. Understanding the underlying biological mechanisms of the effects of Pb, Sb and Mn on preeclampsia may need our advances in knowledge of the pathogenesis of preeclampsia. The study, however, have some flaws. First, as preeclampsia develops in 6–18 weeks of gestation (Redman and Sargent, 2001), blood levels of metals should have been measured at earlier stage of pregnancy. Second, because of developing preeclampsia, renal and hepatic functions are changed (Cunningham et al., 2001). Therefore, these disorders could have influenced blood metal concentrations in preeclamptic subjects. Third, although genetic factor was cited as the risk factors for preeclampsia (Cunningham et al., 2001), because of unreliability of the recall of family history of preeclampsia, this study could not analyze this factor. Further epidemiological studies on larger number of subjects are needed to confirm the findings in the present study.

Acknowledgments

The authors would like to thank of Mr. Christopher Holms, Office of International Academic Affaire, Faculty of Medicine, University of Tokyo, for revising English manuscript, and Mr. Yoshiaki Nakajima, Tokyo Rosai Hospital, for technical assistance. The study was supported by the Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science and partly by the University of Tokyo AGS (Alliance for Global Sustainability) project.

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