Increasing of Syncytial Knot and Fibrinoid Deposit in High-Cd Accumulated Human Placentas

Aumento del Nodo Sincicial y Depósitos de Fibrinoide en Placenta Humanas con Alta Acumulación de Cadmio

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SUMMARY: A toxic metal, cadmium (Cd), can accumulate in human organs. Placenta is usually used as indicator organ for Cd exposure. Therefore, we aim to investigate the different of placental morphology between the low- and high-Cd accumulated placentas. The samples were collected from 14 pregnant women who resided in low-Cd contaminated (L-Cd group) and high-Cd contaminated (H-Cd group) areas. The concentrations of Cd in blood (B-Cd), urine (U-Cd) and placentas (P-Cd) were measured by ICP-MS and AAS. The morphological appearance of placentas was examined by using routine paraffin section and H & E staining. The results showed that levels of B-Cd, U-Cd and P-Cd were significantly higher in H-Cd group than in L-Cd group (p=0.001). Moreover, the B-Cd was positively correlated with U-Cd (rs=0.823, p=0.000) and P-Cd concentrations (rs=0.854, p=0.000). The appearances of syncytial knot (STK) and fibrinoid deposit (Fd) were obviously greater in H-Cd group than in L-Cd group (p=0.007, p=0.026). The STK was positively correlated with both Fd (rs=0.572, p=0.032) and P-Cd concentration (rs=0.766, p=0.001). Although the chorioamnitis and decidual inflammation features were found in both groups but the appearance in H-Cd group seems to be more severe than in L-Cd group. From these results, we suggested that high Cd level in placenta may be involved in morphological changes, especially STK and Fd increasing and probably disturb the connection between maternal and fetal circulation.

KEY WORDS: Cadmium; Placenta; Syncytial knot; Fibrinoid materials.

INTRODUCTION

Cadmium (Cd) is a toxic metal that naturally found in the earth’s crust. The usage of phosphate fertilizer in agriculture and usage of Cd in industry result in the widespread dispersion of this metal into the environment and human foodstuffs (Satarug et al., 2003). In addition, cigarette smoke has been reported as the largest source of Cd exposure in the smoker (Zalups & Ahmad, 2003). Even though the Cd uptake was detoxified by the metal binding protein such as metallothionein (MT) but the high level of Cd uptake was able to accumulate in various human organs (Sakulsak, 2012). In human and animal studies, Cd accumulation induced many adverse effects on various organs such as liver, kidney as well as placenta (Samarawickrama & Webb, 1979; Osman et al., 2000; Kuriwaki et al., 2005; Kippler et al., 2010).

Placenta is a developed organ during pregnancy for protection and supporting fetal growth. However, placenta is considered as an indicator organ for heavy metal exposure (Sorkun et al., 2007). Many studies revealed that Cd accumulation in human placenta involved in morphological changes of placenta and fetal development. For example, alterations of intervillous space, fetal capillaries, villous membrane thickness in placental tissues, and low birth weight neonate were noted in high Cd-contaminated placenta of pregnant smoker (Bush et al., 2000). Additionally, an
aggregation of syncytiotrophoblast (STB) cells which is called syncytial knot (STK) is considered as Cd-induced morphological changes (Sorkun et al.; Akbulut et al., 2009) which is resulted from hypoxia (Heazell et al., 2007). Moreover, other histological findings such as fibrinoid deposit (Fd) (Kaufmann et al., 1996; Bane & Gillian, 2003), and polymorphonuclear (PMN) cell infiltration in chorionic plate (histological chorioamnionitis; HCA) (Murtha et al., 2002; Redline et al., 2003) and in basal plate (decidual inflammation) were considered as histopathologies in placenta as well. Furthermore, Cd has pro-inflammatory properties, which induce chemokine IL-8 recruiting neutrophil and macrophage (Olszowsk et al., 2012). It might be possible that Cd can induce these morphological changes. Therefore, we aimed to investigate the effects of environmental Cd exposure on morphological changes in human placenta.

MATERIAL AND METHOD

Participants and sample collections. This study was conducted under the human ethics committee of Naresuan University, Thailand. Fourteen healthy pregnant women, who resided in Mae Sot sub-district, Tak province and registered at antenatal care units of Mae Sot general hospital between October 2010 and February 2011, were recruited to take part in the study. The pregnant woman who had smoking and alcohol drinking habits was not allowed to be a participant. Then, they were divided into 2 groups according to the placental Cd level; L-Cd group < 15 mg/kg and H-Cd group ≥ 15 mg/kg. Then, maternal blood and urine were collected for Cd measurement at 36 weeks of gestational age. After delivery, the full term-placentas and cord blood were collected immediately and placental weight was measured. Then, central parts of all placentas were collected according to previous study (Kippler et al.). The samples were kept in metal-free cryotubes at -20°C for 20 min. These samples were cooled down at room temperature and adjusted the volume by deionized distilled water to 25 ml. Then, the P-Cd, were measured by inductively coupled plasma mass spectrometer (ICP-MS) (Agilent, Japan).

The measurements of Cd concentrations in blood, urine, placenta and cord blood. For placental Cd concentration (P-Cd) measurement, standard solutions were prepared from Cd-ICP standards (Merck, Germany). One g placental tissues were added with 5 ml nitric acid and were digested at 200°C for 20 min. These samples were cooled down at room temperature and adjusted the volume by deionized distilled water to 25 ml. Then, the P-Cd, were measured by inductively coupled plasma mass spectrometer (ICP-MS) (Agilent, Japan).

Fifty µl blood, cord blood and urine were added by 200 µl matrix modifier containing 0.05 mg NH4H2PO4 and 0.003 mg (NO3)2 (Merck, Germany). Deionized distilled water was used as the blank. The blood Cd (B-Cd), cord blood Cd (C-Cd) and urinary Cd (U-Cd) concentrations were analyzed by graphite furnace atomic absorption spectrophotometry (GF-AAS) (Agilent, Japan).

Histological examination. After tissue processing, placental samples were cut into 5µm-thickness. The deparaffinized and rehydrated sections were performed through xylene and graded ethanol series. Then, the placental sections were stained with hematoxylin and eosin dyes. Finally, the histological changes in the placenta were observed under light microscope (Eclipse 80i, Nikon) and the photographs were taken for further analysis by Image J software base on NIH.

The data were analyzed by SPSS 15.0 for windows evaluation version software. The independent t-test and Mann-Whitney U-test were used for statistical different determination between 2 groups. In addition, the relations between 2 variables were analyzed by spearman’s rank correlation. The data were shown in Mean±SEM the statistical significance level was set at p-value ≤ 0.05.

RESULTS

Maternal and neonatal characteristics. General maternal characteristics were represented in Table I. The mean ages of pregnant women in H-Cd and L-Cd groups were 25.00±2.24 and 25.83±2.78 years, respectively (p=0.836). Additionally, mean body weights, heights and body mass index (BMI) in both groups were not significantly different (p>0.05). The mean parities, number of children, in L-Cd and H-Cd groups were 2.00±0.69 and 1.71±0.36, respectively (p=1.000). Moreover, mean living times in L-Cd group was similar to in H-Cd group (p=0.710). Furthermore, the mean placental weights were 512.50±31.46 and 525±32.27 g in L-Cd and H-Cd groups, respectively (p=0.886).

The neonatal characteristics were represented in Table I as well; the mean gestational ages of both groups were approximately 39 weeks. There were no differences in mean birth length, head and chest circumferences of infants in both groups (p>0.05). The mean birth weight of infants were not statistically different between 2 groups (3,114.29±155.35 and 2,951.42±85.81, p=0.530).

The Cd concentrations in blood, urine, placenta and cord blood. As shown in Table II, the B-Cd and U-Cd concentrations in H-Cd group was remarkably higher than
in L-Cd group approximately 3.5 and 4 folds (p=0.001). The increasing of B-Cd concentrations were obviously associated with U-Cd concentration (rs=0.823, p=0.000). As we expected, P-Cd concentration of H-Cd group was significantly higher than of L-Cd group (p=0.001). The P-Cd levels were positively related with B-Cd and U-Cd as well (rs=0.854, p=0.000 and rs=0.743, p=0.002). In addition, the C-Cd level in H-Cd group was much higher than in L-Cd group about 3 folds and directly related with P-Cd concentrations (rs=0.721, p=0.044).

**Morphological changes in Cd-exposed placenta.**

Histological chorioamnionitis (HCA) is an infiltration of polymorphonuclear (PMN) leukocyte in fetal membrane; either amnion or chorion of placenta (Edwards, 2005). According to the study of Redline and colleagues (Redline et al.), HCA was classified into 3 stages following; stage I is PMN infiltrations in subchorionic plate or few scattered in the lower half of chorionic plate (Fig. 1A). Stage II is diffuse-patchy PMN in fibrous chorion and/or amnion (Fig. 1B). And stage III is PMN karyorrheis or amniocyte necrosis. In our study, the stage I HCA was 80% (4/5) in L-Cd group and 25% (1/4) in H-Cd group. Additionally, stage II HCA in the chorionic plate was found 20% (1/5) in L-Cd group and 75% (3/4) in H-Cd group (Fig. 1G). From these findings, they seem to be more HCA severity in placenta of H-Cd group than in L-Cd group.

In addition, we have found that the mean number of STK/villous area in H-Cd group was remarkably greater than in L-Cd group approximately 2 folds (p=0.007) (Fig. 1C, D and H). Additionally, the increased STK in placenta was positively related with P-Cd concentration (rs=0.766, p=0.001). Moreover, fibrinoid deposit (Fd), an acellular and histologically glossy material was regularly found in placenta. However, it has reported that it was one of pathological findings in the placenta. The Fd replaces the blood clotting after STB degeneration (Kaufmann et al.). The mean Fd area/villous area in H-Cd group (Fig. 1D) was significantly enlarger than in L-Cd group (p=0.026) (Fig. 1C). Interestingly, the Fd in placenta was positively associated with STK (rs=0.572, p=0.032).
According to the classification of HCA (Redline et al.), we have created the stage of decidual inflammation, PMN infiltration into basal plate, based on area of infiltration as followed: Stage I is scattered PMN infiltration in basal plate (Fig. 1E) whereas the PMN infiltration in basal plate and villus along the basal plate is classified as stage II (Fig. 1F). The results were found that the stage I is not found in H-Cd group whereas been shown in L-Cd group about 50%. The stage II is more prominent in H-Cd group (5/5 or 71.43%) than in L-Cd group (2/4 or 50%) as shown in Figure 1G.

**DISCUSSION**

We have revealed that the high B-Cd and U-Cd levels were found in pregnant women who had high-Cd level in placentas. These B-Cd and U-Cd reflect current and long-term exposures to this metal, respectively. These non-smoking women may be exposed to Cd via food, especially rice as same as been suggested by several studies (Osman et al.; Satarug et al.; Kippler et al.). Because rice is a main food of
Thai people and is reported to be contaminated with Cd exceeding the provisional maximal level of 0.2 mg/kg (Simmons \textit{et al}., 2005). Therefore, the person who mainly consumed rice grown in the Cd-contaminated area had higher U-Cd than those who did not (Swaddiwudhipong \textit{et al}., 2007).

After Cd uptake, it is mainly accumulated in target organs, liver and kidney (Kuriwaki \textit{et al}.). However, other organs including placenta have been reported to retain Cd which is used as indicator organ for Cd exposure (Bush \textit{et al}., 2000; Osman \textit{et al}.; Sorkun \textit{et al}.). Interestingly, all pregnant women who had high P-Cd level also reside in Mae Ku, Mae Tao and PrathatPhadeang where were reported as high Cd-contaminated areas (Simmons \textit{et al}.). This finding was in agreement with the studies in Swedish, Turkish and Bangladesh pregnant women who living in air polluted and Cd-contaminated areas (Osman \textit{et al}.; Sorkun \textit{et al}.; Kippler \textit{et al}.).

This Cd accumulation was reported to alter the morphology and function of placenta relating neonatal interference. The morphological appearance of STK in our study was similar to previous study that reported an increased interference. The morphological appearance of STK in our study. The Fd is derived from blood clotting or degenerative seen in association with Fd (Heazell \textit{et al}.) that was similar to

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We are grateful for Commission on Higher Education (CHE) funding under Strategic Scholarships for Frontier Research Network for the Joint Ph.D. Program. And we would like to express our sincere thanks to all staffs of Mae Sot General Hospital and toxic metal research group (TMRG), Naesuan University for collaboration and samples collection.


RESUMEN:

Un metal tóxico, el cadmio (Cd), se puede acumular en órganos humanos. La placenta se utiliza, por lo general, como órgano indicador de la exposición a Cd. Nuestro objetivo fue investigar la diferente morfología placentaria entre las placenta con baja y alta acumulación de Cd. Las muestras fueron recolectadas de 14 mujeres embarazadas que residían áreas con alta (grupo H-Cd) y baja contaminación por Cd (grupo L-Cd). Las concentraciones de Cd en la sangre (B-Cd), orina (U-Cd) y placenta (P-Cd) se midieron por ICP-MS y AAS. La apariencia morfológica de las placenta fue examinada usando cortes histológicos teñidos con H-E. Los resultados mostraron que los niveles de B-Cd, U-Cd y P-Cd fueron significativamente mayores en el grupo H-Cd (p=0,001). Por otra parte, el B-Cd se correlacionó positivamente con las concentraciones de U-Cd (rs=0,854, p=0,000) y P-Cd (rs=0,823, p=0,000). Las apariciones de nodos sinciciales (NS) y depósitos fibrinoide (Fd) fueron mayores en el grupo H-Cd (rs=0,007, p=0,026). Los ND se correlacionaron positivamente con los Fd (rs=0,572, p=0,032) y la concentración de P-Cd (rs=0,766, p=0,001). Aunque características de corioamnitis e inflamación de la decidua se encontraron en ambos grupos, su aparición en el grupo H-Cd pareció ser más grave que en el grupo L-Cd. A partir de los resultados, sugerimos que el nivel alto de Cd en la placenta puede estar involucrado en los cambios morfológicos, especialmente el aumento de NS y Fd, los que probablemente alteran la relación entre la circulación materna y fetal.

PALABRAS CLAVE: Cadmio; Placenta; Nodo sincicial; Materiales fibrinoide.
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Received: 09-07-2013
Accepted: 28-08-2013