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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

Laorrat Phuapittayalert[*]; Thongchai Norkaew[**]; Wisa Supanpaiboon[***]; Supaporn Chuenchoojit[****]; Wiphawi Hipkeao[*****]; Witaya Swaddiwudhipong[******] & Natthiya Sakulsak[*]

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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 wide-spread 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

[*] Department of Anatomy, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand.
[**] Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand.
[***] Department of Biochemistry, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand.
[****] Division of Community Public Health, Faculty of Science, BurirumRajabhat University, Burirum, Thailand.
[*****] Department of Anatomy, Faculty of Medicine, KhonKaen University, KhonKaen, Thailand.
[******] Department of Community and Social Medicine, Mae Sot General Hospital, Tak, Thailand.

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 (Olszowski *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 -20C for Cd analysis and were fixed in 4% paraformaldehyde in 0.1M phosphase buffer saline pH 7.4 for histological examination.

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 $NH_4H_2PO_4$ and 0.003 mg $Mg(NO_3)_2$ (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

Table I. The characteristics of mother and neonate.

Variables	L-Cd group (n=7)	H-Cd group (n=7)	*p*-value
Maternal characteristics			
Age (year)	25.00±2.25	25.83±2.78	0.836
Body weight (kg)	66.57±4.80	63.42±4.46	0.945
Height (m)	1.57±0.02	1.56±0.30	0.732
BMI (kg/m^2)	31.64±2.03	26.03±1.20	0.836
Parity	2.00±0.69	1.71±0.37	1.000
Living time (years)	25.00±2.254	25.71±3.49	0.620
Placental weight (g)	512.50±31.46	525±32.27	0.886
Infant characteristics			
Gestational age (week)	39.43±0.52	39.40±0.87	1.000
Birth weight (g)	3114.29±155.35	2950.00±100.00	0.530
Birth length (cm)	51.71±0.99	51.60±0.93	0.876
Head circumference (cm)	32.36±0.56	33.40±0.81	0.343
Chest circumference (cm)	33.50±0.93	31.80±0.49	0.343

Table II. The Cd concentrations in blood, urine, placenta and cord blood.

Cd concentrations	L-Cd group	H-Cd group	*p*-value
Blood Cd (µg/L)	0.49±0.05	1.76±0.37	0.001**
Placental Cd (µg/kg)	9.42±0.87	34.88±8.79	0.001**
Cord blood Cd (µg/L)	0.17±0.05	0.58±0.22	0.114
Urinary Cd (µg/g creatinine)	0.84±0.05	3.15±1.10	0.001**

** $p<0.01$

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

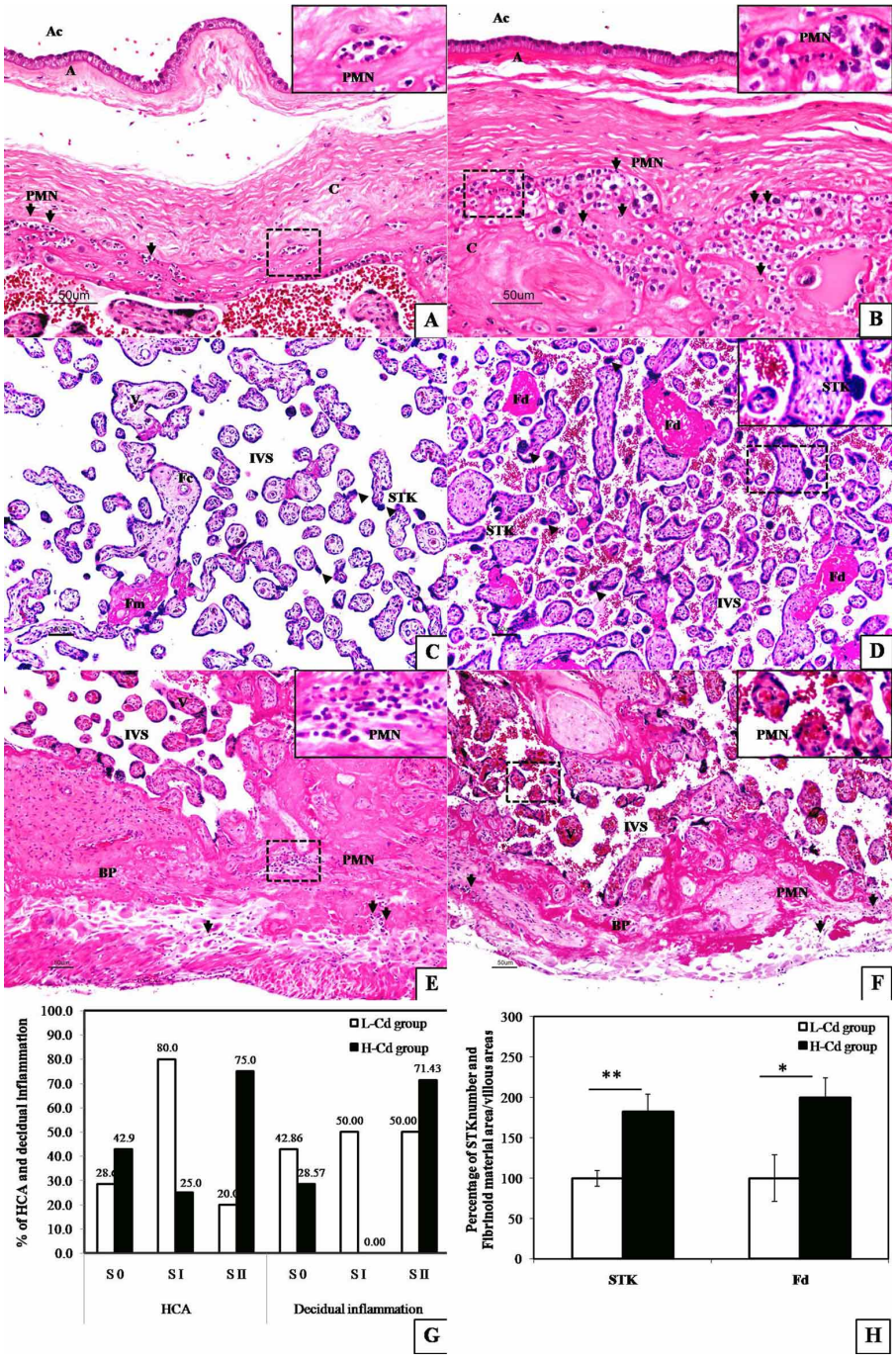

Fig. 1. Morphological change in human placenta (A-F), the quantitative analysis of HCA and decidual inflammation (G) and of STK and Fd (H):Infiltration of PMN cells (arrow) in subchorion or stage I of HCA (Fig A);Infiltration of PMN cells in Chorion or stage II HCA (Fig B). The numbers of syncytial knot (STK) and fibrinoid deposit (Fd) were smaller in L-Cd group than in H-Cd group (fig C and D). The stage I decidual inflammation was shown in Fig E whereas the stage II decidual inflammation with villitis along basal plate (BP) as shown in Fig F. The quantitative analysis of HCA and decidual inflammation found that the severity of these morphological features tend to be occurred in H-Cd group than in L-Cd group (Fig G). The quantitative analysis showed that the STK and Fd were greater in H-Cd group than in L-Cd group ($p=0.007$, 0.026; $*p<0.05$, $**P<0.01$) as shown in Fig H. Ae=amnionic epithelium; Am= amnionic mesoderm; Sl= spongy layer; Cm= chorionic mesoderm; Bm= basement membrane; Lf= Langerhans' fibrinoid stria; IVS= intervillous space; V= chorionic villus; STK= syncytial knot; Fd= fibrinoid deposit.

Thai people and is reported to be contaminated with Cd exceeding the provisional maximal level of 0.2 mg/kg (Simmons *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 *et al*., 2007).

After Cd uptake, it is mainly accumulated in target organs, liver and kidney (Kuriwaki *et al*.). However, other organs including placenta have been reported to retain Cd which is used as indicator organ for Cd exposure (Bush *et al*., 2000; Osman *et al*.; Sorkun *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 *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 *et al*.; Sorkun *et al*.; Kippler *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 STK in the placenta of pregnant women who resided in air polluted area (Sorkun *et al*.). An excessive number of STK in placenta resulted in inadequate fetal circulation due to maternal flow obstruction at the group of these nuclei (Jones & Fox, 1977). Furthermore, this STK was formed after various conditions such as hypoxia, hyperoxia and ROS exposure (Heazell *et al*.). The features of STK nuclei are classified by pyknosis, peripheral chromatin condense and fusion of cell membrane which is similar to those described in apoptosis (Heazell *et al*.). Additionally, Cd involves in mitochondria, caspases, and ROS pathways, all seems to play a role in Cd-induced apoptosis (Pulido & Parrish, 2003). Therefore, we suggested that Cd may be involved in the STK formation in the placenta. Moreover, the increased STK was frequently seen in association with Fd (Heazell *et al*.) that was similar to our study. The Fd is derived from blood clotting or degenerative processes of STB (Kaufmann *et al*.). The study of glycosis blockage caused STB degeneration and immediately followed by blood clot at the trophoblast surface (Kaufmann *et al*.). The increased accumulation of Fd at denudation site of placenta was also reported in smoker (Mayhew *et al*., 2003). From our results, we suggested that the increased Fd probably occurred to replace the injury site after STB apoptosis.

About other morphological changes, the feature of HCA and decidual inflammation were PMN infiltration. In general, the most cases of HCA can occur with no any clinical signs. Moreover, twenty percent of HCA can be found in term deliveries whereas upto 50% can be found in pre-term deliveries (Edwards, 2005). However, all pregnant women in this study were full-term delivery. Although HCA were not much different between 2 groups, it seems to be more severity of HCA and decidual inflammation in H-Cd group than in L-Cd group. Previous study was reported that Cd has pro-inflammatory property by activates IL-8 resulting in recruiting neutrophil and macrophages after Cd exposure (Olszowski *et al*.). Hence, Cd may involve in PMN infiltration in the placenta.

In this study, we suggest that Cd is likely entering the human body by food chain. Then, it can be accumulated in many organs including placenta and probably induced morphological changes in placenta, especially STK and Fd formations. These STK and Fd appearances may further disturbed the fetomaternal circulation. Then, the apoptosis in Cd-accumulated placenta should be further investigated.

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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 placentas 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 placentas (P-Cd) se midieron por ICP-MS y AAS. La apariencia morfológica de las placentas 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 fibrinoides (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 estos 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 fibrinoides.

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Correspondence to:
Natthiya Sakulsak, PhD
Department of Anatomy
Faculty of Medical Science
Naresuan University
Phitsanulok, 65000
THAILAND

Email: nsakulsak@yahoo.com

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