CHAPTER V

DISCUSSION

We investigated Cd concentration in maternal blood, urine, placenta and umbilical cord of pregnant participants. The Cd levels of maternal blood and placenta in pregnancies who live in Cd contaminated area were significantly higher than those in non-Cd contaminated area about 2.1 and 2.2 times, respectively ($p<0.05$). Moreover, the statistical analysis showed the strongly positive correlation between the Cd in maternal blood and placenta ($p=0.0001$). Thus, the relative high Cd concentration in placenta could be explained by the high Cd concentration in maternal blood. One of the main sources of Cd exposure in the general population is tobacco smoke, and it had been reported that smoking pregnant woman increased Cd in blood and placental body burden (Kuhnert, et al., 1987; Piasek, et al.,. 2001). Moreover, the Cd accumulation was found in human placenta as shown in the result of this study. Thus not only liver and kidney, but placenta is also an organ where to accumulated Cd uptaken as reported in the previous study (Osman, et al., 2002). These suggested that women living in Cd contaminated area have a high risks for Cd uptaken and accumulation in several organs of body (Kim, et al., 2007).

The Cd levels in urine or urinary Cd and in umbilical cord blood were not significantly different between pregnancies who live in non-Cd and Cd contaminated areas. However, the urinary Cd in pregnancies who live in Cd contaminated area was 1.4 times more than those in non-Cd contaminated area. And, this mean value was about 2.20±0.41 μg/g creatinine which over than the biological index limitation for environmental Cd exposure, 2 μg/g creatinine (WHO, 2000b). In a study of Thai population without inordinate Cd exposure proved that urinary Cd excretion was usually < 2 μg/g creatinine (ATSDR., 1999, Sirivarasai, et al., 1992). Indeed, subjects who had urinary Cd excretion exceeded 2 μg/g creatinine were significantly increased predominance of calciuria (Wu, et al., 2001). The previous study, in women who received Cd from dietary source about 90 μg/day, found that their urinary Cd were

exceed 2 μg/g creatinine. Whereas, in women who received dietary Cd contamination less than 30 μg per day (< 30 μg/day), their urinary Cd were less than 1.4 μg/g creatinine (Satarug, et al., 2004). The Cd concentration in umbilical cord blood of pregnancies who live in Cd contaminated area was higher than those in non-cd contaminated areas about 2.5 times. This result was consistency with the previous study in rural pregnant Bangladeshi (Kippler, et al., 2010). They found that the Cd in placenta was positively associated with Cd in umbilical cord blood.

In addition, we also found the strongly positive correlation between Cd in maternal blood and in placenta (*p=0.0001). In general, blood Cd means the best dose estimate of acute exposure, whereas urinary Cd means a useful estimation for kidney or body burden which is long term exposure (Järup, et al.. 1998). And, women usually have higher cadmium body burden than men (Nishijo et al., 2004; Vahter et al., 2007). Previous study indicated that there was a good association between current urinary Cd and blood Cd ($r^2 = 0.879$) ($p < 0.0001$) (Järup, et al., 1997).

The serum ferritin and placental Fe levels were not different between both non-Cd and Cd contaminated groups. These may because all pregnant participants received the same dosage of iron supplement providing from Ministry of Public Health. And, an essential Fe element is needed for fetal growth and development so that it usually passes through placental barrier and final enters fetal capillaries. Interestingly, we found that the placental Fe trended to have an inverse relationship with the placental Cd. This may because both Cd and Fe elements are divalent metal and it has been reported that they share the same transporters such as DMT-1 (Rolfs A., et al 2002: Elisma and Jumarie, 2001). And, their transport characteristics are competitive binding to the transporter binding site (Elisma and Jumarie, 2001). Although, we found the high level of Cd accumulation in placentas taken from pregnancies who live in Cd contaminated area but the fetal birth weight of both non-Cd and Cd contaminated groups were similar. This may explain that the acute sign of Cd toxicity usually occur when received Cd in high dosage (Waalkes, et al., 1992) and the chronic sign is detected after prolong Cd uptake in several years (Norberg, et al., 2004; Satarug, et al., 2004). However, we also found the Cd contaminated in the fetal cord blood (data not shown). The previous study by Sonawane and coworkers, 1975 found high Cd accumulated in fetal rat after maternal Cd administration (Sonawane,

et al., 1975). And, they suggested that Cd acrossed the placenta. However, cord blood was about 10% of maternal blood Cd (Kuriwaki, et al., 2005). Then, this finding indicated that the infants were an early life dosage of Cd concentration eventhough they have no any system.

The localization of DMT-1 in fetal portion of placenta, we found that DMT-1 localized in apical surface of cytoplasm and basal surfaces of STB as well as cytoplasm of the endothelial cells of fetal capillary and hofbuaer cell. According to previous study in the rat intestinal found that DMT-1 is an apical membrane Fe transporter in intestinal epithelial cells (Fleming, et al., 1999) and uptake Cd from intestinal lumen (Griffith, el al., 2000 ; Tallkvist, et al., 2002). We suggested that DMT-1 on apical surface of cytoplasm of STB also uptake divalent metal from maternal circulation. The result were consistently with previous study which reported that DMT-1 was localized both in the cytoplasm as well as the junction of the basal membrane and fetal vessels and macrophage cell in placental tissue (Georgieff, et al., 2000). Hence, we suggested that it was responsible for transportation of Fe or Cd across endosomal membrane into cytoplasm of these cells including STB, endothelium and macrophage in a manner similar to enterocyte of duodenum (Griffith, el al., 2000, Tallkvist, et al., 2002). The DMT-1 at the basal membrane interface with fetal vessels implies a role for DMT-1 in the export of iron across that membrane (Georgieff, et al., 2000). Therefore, DMT-1 may also export Cd by passing the basal membrane of STB. In maternal portion of placenta, we found DMT-1 localized in cytoplasm of most decidaul cells. This may implied that DMT-1 acts as divalent metal transporter in decidual cells as same as in STB. In addition, it had been suggested that Cd was accumulated in decidual cell of placenta by detected of MT expression in these cells (Goyer, et al., 2004). Hence, we suggested that DMT-1 in placental decidual cell may involve in Cd transport into this cell. Moreover, we found DMT-1 localized in placental barrier or membrane which composes of cytoplasm of STB and endothelium of the fetal capillary. This area is semipermeable layer of tissue separating maternal blood from fetal blood in the placenta (Gude, et al., 2004). In general, this placenta membrane allows the nutrients and essential metals such as Fe and Zn, that are necessary for fetal growth and development passing through and enters to the fetal circulation (Carlson, et al., 1999; Gude, et al., 2004). Unlike the essential metals, Cd

is a non-essential metal element which has only very limited placental transfer and it is sequestered in the placenta during pregnancy (Goyer and Cherian, 1992). Hence, we suggested that DMT-1 obviously exists and functions on the placental membrane. It has been documented that DMT-1 localized in subapical and intracellular in hepatocytes and suggested the importance of DMT-1 in Fe and Cd transport in rat kidney (Smith and Thévenod, 2009). Thus, DMT-1 likely involve in several divalent metal transport including Cd.

We investigated amount of DMT-1 positive immunoreactivity in STB in different areas in placenta including cord insertion, central and marginal areas. The different placental areas where it anatomical structures of blood vessels are differences. This result showed that DMT-1 was predominantly localized in insertion area followed by central and marginal areas, respectively. Thus, the insertion area likely has a function in transportation of essential and non-essential by DMT-1 more than the other areas, central and marginal areas. When comparing between low-Cd and high-Cd groups, the numbers of STB which expressed DMT-1 positive immunoreactivity, were not different. However, it seemed like that the expression intensity of DMT-1 positive cell in high-Cd group was more intense than in low-Cd group. To define the differential DMT-1 protein expression, we also performed the Western blot analysis.

According to Western blotting analysis, DMT-1 protein expression in high-Cd was significantly higher than low-Cd about 1.19 times. It has been reported that Pb and Cd exposure in the induced DMT-1 protein synthesis in developing rat brains (Gu. et al., 2008). In addition, previous finding represented the affinity ranking of DMT-1 for metal ions transport as Mn > Cd? > Fe? > Pb ~ C~ Ni >Zn (Garrick et al., 2006). These studies showed that the Km of Mn^{2+} and Fe^{2+} for DMT-1 were ~1 μM and ~3 μM, respectively (Garrick, et al., 2006). DMT-1 had a lower affinity of Fe^{2+} than Cd^{2+}, the *K*m value of DMT-1 for Fe^{2+} was approximately 2 μM at pH 5.5 in mammalian cells (Gunshin, et al., 1997). The averaged *K*m value of DMT-1 for Cd was 1.04 ± 0.13 μM (Okubo, et al., 2003). Then, an unnecessary DMT-1 protein increasing may serve for Cd transportation in placental tissue and accumulated in the placenta as we found the high-Cd level in the placentas taken from pregnancies who live in Cd contaminated area. Because we also found Cd in cord blood of both non-Cd and Cd

contaminated groups (data not shown). Then, there is a high possibility that Cd can further pass through placental barrier via the DMT-1 transporter and enters the fetal capillary (Bressler, et al., 2004; Elisma and Jumarie, 2001).

Consistency with the result of DMT-1 protein expression, DMT-1 mRNA expression in high-Cd was significantly higher than in low-Cd about 1.34 times. Under Cd exposure condition, there are many reasons to support that DMT-1 may be activated in level of gene transcription and translated to protein. It has been documented that Cd influenced the increasing in protein and mRNA expressions in the duodenum as well as other peripheral tissues (Kim, et al., 2007; Gu, et al., 2008). The study in knockdown clonal DMT-1 in Caco-2 cell lines found the decreasing in DMT-1 mRNA levels. Moreover, Fe and Cd transports were also reduced and showed competition between Cd and Fe uptake. They proposed that DMT-1 mediated the transport of Cd (Desmond, et al., 2003). Our result found the DMT-1 increasing together with a high Cd level in placentas taken from Cd contaminated group whereas the Fe level in placentas trended to be decreased. These conditions were similar as the study of Cd effects on DMT-1 expression in intestinal rat. It showed that Fe deficient diet was increased Cd accumulation as well as DMT-1 and MTP-1 expressions in the duodenum. However, the Cd accumulated, DMT-1 and MTP-1 expressions returned to control levels when the rats were ingested Fe sufficient for 4 weeks. These results suggested that DMT-1 and MTP-1 involved in Cd absorption in duodenum rat (Ryu, et al., 2004). The relationship between Cd absorption and DMT-1 expression were observed in pregnant rat and suggested a role for DMT-1 in the increased absorption of Cd during pregnancy (Leazer, et al., 2002). Another previous study, they investigated that DMT1 IVS4+44C/A single nucleotide polymorphism (SNP) has an effect on Fe, Zn essential trace element levels and Pb, Cd toxic metals accumulation in the placenta samples. These result represented no significant association between the IVS4+44C/A SNP in the DMT-1 and Pb, Zn, and Fe levels in the placenta samples but statistically significant association was detected with the Cd concentration (Gayaalti, et al., 2010).

The mechanism of DMT-1 transporter has been proposed in several previous studies. They investigated the 5' regulatory region on human nramp2 and reported that nramp2 also contains five potential metal response elements (MRE's) that similar to

nramp2 also contains five potential metal response elements (MRE's) that similar to the MRE's was found in the MT-II_A and ZnT1 genes (Lee et al., 1998). The present of Cd can induced MT-II_A and ZnT1 genes transcription in these MRE's element (Martelli et al., 2006). Another metal transcription factor (MTF-1), an intracellular Zn level controller, is activated by cellular Zn level. After activated by Zn overloading, Zn-MTF-1 complex translocated to the nucleus and binds to MRE's on MT-II_A and ZnT1 resulting in MT-II_A and ZnT1 genes transcription (Andrew et al., 2001). It has been suggested that Cd can induce MTF-1 translocatation by the displacement of Zn (Zhang et al., 2003). Therefore, we proposed that Cd accumulated in placenta may also induce MTF-1 function to activate its transportation and bind on MRE's element which found in DMT-1 gene and further resulting in DMT-1 transcription.

According to the passage, we suggested a possible pathway of DMT-1 transcription in STB of human placenta as shown in figure 45. These may cause cellular damage in placenta especially in placental membrane where interrupt its function then, Cd may be transported the fetus.

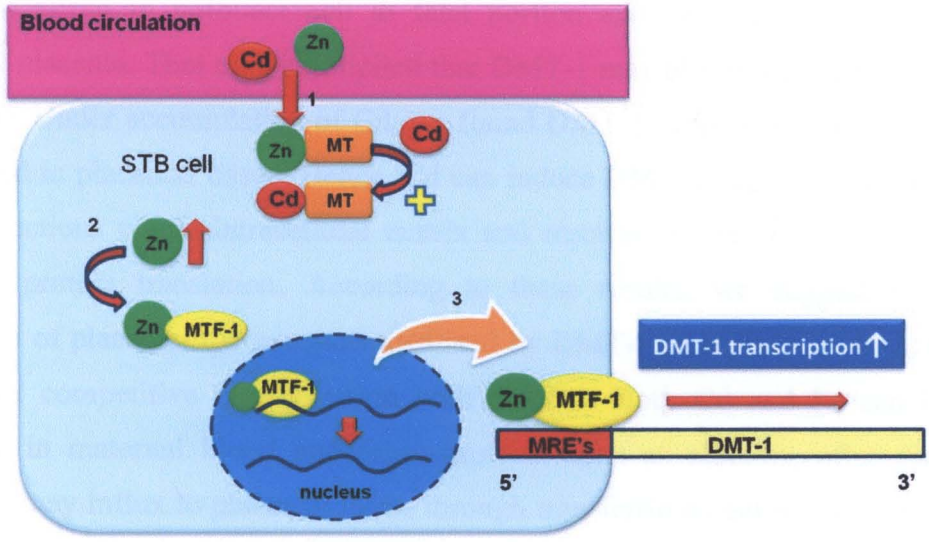

Figure 45 A possible pathway of DMT-1 transcription under high placental Cd accumulation. 1; Cd and Zn were uptaken in intracellular matrix and bound to MT, and Cd is high affinity to MT more than Zn. (2) Increasing intracellular Zn concentration, Zn bounds MTF-1 resulting in activate DMT-1 transcription. (3) MTF-1 binds MRE's containing in promoter of DMT-1 gene and facilitates DMT-1 transcription.