

OSTEOTOXICITY IN THE INHABITANTS OF A CADMIUM POLLUTED AREA

Kowit Nambunmee*

Major of Public Health, School of Health Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

ABSTRACT: Cadmium (Cd) is a toxic metal which causes osteotoxicity including osteopenia, osteoporosis and osteomalacia. Itai-itai patients were the best known typical victims of Cd intoxication in Jinzu river basin showing osteomalacia, severe bone pain, and high risk of bone fracture. These patients had been declared as having the most severe osteotoxicity caused by Cd exposure. With the growing use of Cd in industry, more populations at risk of Cd osteotoxicity have been identified. A model which explains the mechanism of how Cd affects the bone will have an application for the management and prevention of Cd osteotoxicity. This paper proposes a model to explain Cd osteotoxicity which is classified into direct and indirect effect. The direct effect relates to an imbalance between bone tissue synthesis by osteoblasts and bone resorption by osteoclasts. Cd induces osteoblast apoptosis, and decreases osteoblast activity disturbing bone tissue synthesis while it enhances bone resorption by osteoclasts causing low bone mass. The indirect effect relates to decreased vitamin D active metabolite production in the kidney, decreased calcium (Ca) absorption from gastrointestinal tract, and increased wasting of Ca into urine by proximal tubular dysfunction. The long term effects of bone cells disturbance and proximal tubular dysfunction cause an imbalance of bone remodeling with enhanced bone resorption and abnormal bone tissue synthesis characteristic of Cd osteotoxicity. The model of Cd osteotoxicity justifies the supplement of vitamin D active metabolite and Ca for the treatment and protection against severe bone pathology.

Keywords: Cadmium, Osteotoxicity, Proximal tubular dysfunction, Itai-itai, Bone metabolism, Calcium wasting

INTRODUCTION

Cadmium (Cd) is a toxic metal which is a by-product from zinc mining and industrial processes. If Cd waste is discharged into the public water irrigation system it can pollute agricultural and living areas, accumulate into the soil and the crops, especially rice and tobacco. The consumption of contaminated crops and tobacco are the main source of Cd exposure among the inhabitants of a Cd polluted area. The absorption rate of Cd via gastrointestinal (GI) tract is 5-8 % whereas the absorption rate of Cd via respiratory tract is higher at 10-50% [1]. Cd polluted areas have been identified in Belgium, Sweden, Japan, China and Thailand in which high level of Cd contamination in soil, rice, tobacco and vegetable were reported [2, 3]. The Cd in soil, and rice in these polluted

areas were higher than the recommended level to prevent potential Cd-induced detrimental health impact (<3.0 mg/kg for soil and <0.2 mg/kg for rice grain [4]).

RISK FACTORS OF Cd EXPOSURE

Factors that increase Cd exposure are smoking, low body storage of iron (Fe), zinc (Zn) and Ca, and pregnancy (due to increased Fe absorption) [2, 3, 5]. Smokers have been found to have higher Cd burden than non-smokers. It has been estimated that 1-2 µg Cd per cigarette is inhaled [5]. Therefore, less smoking in a Cd exposed community would help to reduce the exposure by causing less passive smoking [6].

Dietary Cd is absorbed in the GI tract by a divalent metal transporter (DMT), also a transporter of Fe, Zn, and Ca. Low body storage of these essential metals (Fe, Zn, Ca) stimulates DMT which in turn increases dietary Cd absorption [2, 5].

* Correspondence to: Kowit Nambunmee
E-mail: kowit.nam@mfu.ac.th

During pregnancy the DMT activity increase to facilitate Fe absorption; with increased activity of DMT more Cd is absorbed. Multiple pregnancies was believed to be a contributing factor in the development of Itai-itai disease [6].

PATHOPHYSIOLOGY

The Cd exposure is related to renal tubular dysfunction, anemia, diabetes, hypertension, gall bladder stone, prostate cancer, lung cancer, and bone disease [7, 8]. Cd is accumulated in the liver and kidney with an elimination half-life of 10-30 years [7]. Cd concentration in urine is proportional to its concentration in the kidney and is a useful exposure biomarker. Urinary Cd level above 2.5 µg/ creatinine (Cr) is associated to an excess prevalence of renal tubular damage of 4% and the level above 1 µg/ Cr is associated to an increase of osteoporosis risk [1, 7]. Renal tubular dysfunction and bone diseases are the specific characteristic of Cd intoxication. The most severe Cd intoxication victims are itai-itai patients who had high Cd body burden, severe proximal tubular dysfunction and multiple pseudo-fractures with severe bone pain [9].

Itai-Itai patients

The endemic of Itai-itai was found in Jinzu river basin of Toyama prefecture in the 1920s [10]. Their Cd excretion levels ranged 10-30 µg/g Cr [11] with increased urinary excretion of total protein, low molecular weight protein, amino-N proline, glucose and calcium (Ca). Their bone radiological findings showed Looser's zones; a narrow radiolucency which transects one or both cortical margins of a bone, and is a definite indication of osteomalacia [12]. The proposed mechanism to explain the osteomalacia among these patients was Cd induced renal tubular osteomalacia [10]. The kidneys are the production house of vitamin D active metabolite and Ca reabsorption site. Once renal tubular dysfunction appears hydroxylation in the kidney decreases which reduces the production of vitamin D active metabolites [13, 14]. With the progression of renal tubular dysfunction vitamin D active metabolite production becomes more impaired, parathyroid hormone (PTH) level increases, the absorption of Ca from GI tract decreases, Ca excretion into urine increases resulting in increased bone resorption and finally bone damage [14, 15].

However, the above osteotoxicity mechanism in Itai-itai patients could not explain all the phenomena seen in other Cd exposed people. Honda et al. found decreased bone mineral density in exposed Japanese women without signs of renal

dysfunction [16]. Engström et al. [17] proposed that the Cd induced bone damage was not related to inactivation of vitamin D active metabolites. On the other hand, Jin et al. [18] found increased prevalence of osteoporosis in renal tubular dysfunction group. These informations demonstrated the complexity of Cd induced osteotoxicity which requires further explanation.

This paper proposes a mechanism of osteotoxicity which is classified into direct and indirect mechanism. The direct mechanism involves a disturbance of bone cells causing increased bone resorption, decreased bone formation and decreased calcification. The indirect mechanism involves renal dysfunction which is related to the vitamin D metabolism and wasting of calcium (Ca) into urine.

BONE METABOLISM

Bone is a resource of Ca in human body which is controlled by PTH, vitamin D and calcitonin [19, 20]. Blood Ca is maintained by the balance of the absorption of Ca from the intestine, the excretion of Ca in the urine and the calcification and decalcification of bone [21]. PTH increases serum Ca by increasing renal tubular reabsorption of Ca and bone resorption. Vitamin D increases serum Ca by increasing intestinal absorption of Ca [22]. The most effective form of Vitamin D is $1\alpha,25(\text{OH})_2\text{D}$ which is produced by kidney cells from 7-dehydrocholesterol in the skin and the dietary obtained vitamin D. The hydroxylation of vitamin D in the kidney produces $1\alpha,25(\text{OH})_2\text{D}$ which increases the absorption of Ca from GI tract [23].

At a molecular level, the bone remodeling is a function of osteoblast and osteoclast cells. The activities of osteoblasts and osteoclasts are strictly balanced by extracellular signals to prevent the loss of bone mineral and bone mass [24, 25]. The osteoblast synthesizes bone matrix and deposits Ca to the bone matrix, a process called calcification, while the osteoclast resorbs bone by releasing Ca into the blood circulation called decalcification. Serum Ca is the main signal to control the functions of these cells. Long term Ca wasting and high degree of bone resorption causes bone damage [21, 26]. Cd increases both bone resorption and Ca wasting which are the risks of bone disease.

Cd INDUCED BONE DAMAGE VIA RENAL TUBULAR DYSFUNCTION, THE INDIRECT EFFECT

Kidney is a bone metabolism related organ. It regulates the Ca absorption from urine and the production of vitamin D active metabolite ($1\alpha,25(\text{OH})_2\text{D}$). Renal dysfunction has been found

to be a main cause of bone damage in Cd exposure. The prevalence of osteoporosis, a low bone mass and micro-architectural deterioration of bone tissue with consequent increase in bone fragility and fracture risks, was increased in proportion to the degree of renal tubular damage. In Cd exposed Chinese, osteoporosis prevalence in Cd level 10-20 µg/g Cr was 6.20 % compared to 2.41 % in Cd level <2.0 µg/g Cr group [18]. The prevalence of osteoporosis in exposed females with tubular dysfunction was 2.81 times higher than those without tubular damage [27]. In addition, Aoshima et al. [28] reported that exposed women with urinary Cd means 17.2 µg/g Cr (range 5.7-37.7 µg/g Cr) showed renal dysfunction and impaired osteoid mineralization which correlated with the development of osteomalacia.

The degree of bone remodeling among Cd exposed inhabitants was also related to renal tubular dysfunction. The results of study by Nambunmee et al. [29] indicated a positive correlation between bone markers (serum osteocalcin and urinary N-terminal cross link of type I collagen (NTx)) and proximal tubular dysfunction markers (urinary β₂-microglobulin and fractional excretion of calcium). Horiguchi et al. also reported a significant increase of bone resorption marker (NTx) in exposed group (>3.5 µg/g Cr) when compared with reference group (<2.5 µg/g Cr), and renal tubular dysfunction was observed in exposed population [30]. They suggested that Cd induced bone mineral loss was related to renal tubular dysfunction.

When a vitamin D metabolizing enzyme system in the proximal tubules is disturbed this causes a decrease in vitamin D active metabolite, 1α,25(OH)₂D, production. Several studies of 1α,25(OH)₂D and PTH in Cd exposed inhabitants with renal dysfunction showed the decreased 1α,25(OH)₂D levels were closely correlated to increased PTH and β₂-MG levels [14, 31-35].

Tsuritani et al. [36] found that renal damage due to Cd exposure led to a decrease in the serum 1α,25(OH)₂D level and an increase in serum PTH which is in accordance with the study of Nogawa et al. [34] who showed that renal dysfunction was related to increased serum PTH and Cd-induced bone damage.

The above evidences support a mechanism of proximal tubular dysfunction as the cause of Cd osteotoxicity. The proximal tubule is the major site for essential nutrient reabsorption and it is the site of Vitamin D active metabolite production. Once Cd accumulated in the kidney reaches the threshold level to induce proximal tubular dysfunction, a

decrease of serum vitamin D active metabolite and an increase of Ca wasting was found. The decrease of serum Vitamin D active metabolite causes the decreased reabsorption Ca from GI tract which in turn increases the secretion of PTH. The PTH activates osteoclasts to resorb bone, releasing Ca into blood circulation. Another explanation is the wasting of Ca into urine decreases serum Ca which in turn increases the bone resorption. A long term low vitamin D active metabolite and increased Ca wasting result in the bone being continuously resorbed which causes decreased bone mineral density and osteoporosis.

DIRECT EFFECTS OF Cd ON BONE

In addition to the evidence showing a relation between renal tubular dysfunction and bone pathology in Chinese and Japanese subjects, other studies also showed the clue that Cd may directly disturb bone metabolism. Wallin et al. [37] reported the negative association between bone mineral density and Cd level in Swedish subjects with kidney cortex Cd level at 22.9 µg/g wet weight (ww) which is lower than the level required to show renal tubular dysfunction at 50 µg/g ww. Åkesson et al. [38] determined the effect of low exposure of Cd (Urinary Cd mean 0.52 µg/l) on bone health and they found a negative relation between urinary Cd and bone mineral density. They proposed that Cd decreased bone mineral density directly by causing the bone cells to increase bone resorption, which was shown by increased level of bone resorption marker, and urinary wasting of Ca.

The association between Cd body burden and increased risk of osteoporosis has also been reported in occupational exposure. The subjects with urinary Cd >1.88 µg/g Cr had an osteoporosis risk 9.9 times higher than the group with urinary Cd <0.51 µg/g Cr in whom renal tubular dysfunction was not observed [39].

A direct effect of Cd on bone metabolism showing bone remodeling biomarkers has been demonstrated. Abnormal synthesis of bone tissue was indicated by an increase in bone formation markers, serum osteocalcin [28, 31, 32], and bone alkaline phosphate [40]. Bone resorption-acceleration indicated by high level of deoxypyridioline (DPD), N-terminal and C-terminal crosslink of type I collagen (NTx and CTx) was evident even in low Cd exposed individuals [38, 41, 42]. In addition, the bone resorption acceleration and abnormal bone tissue synthesis found in Cd exposure was shown to be independent of kidney dysfunction [43].

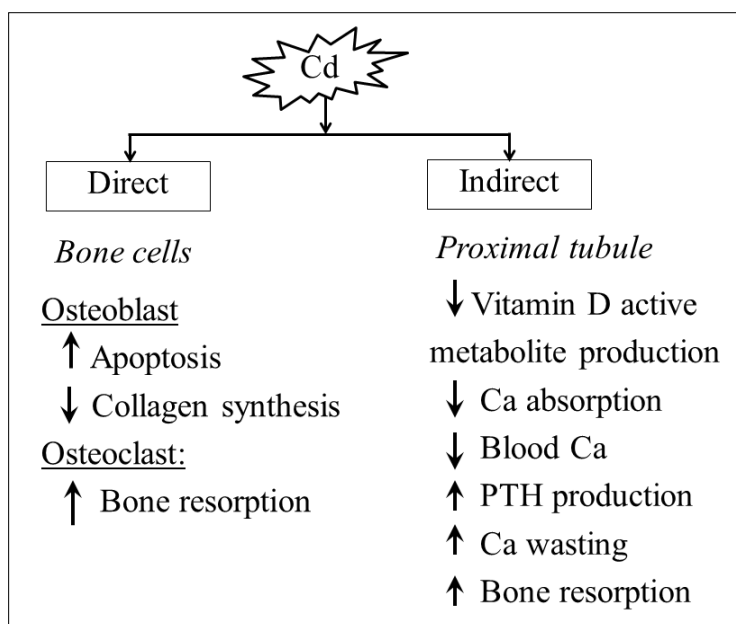


Figure 1 Proposed Cd osteotoxicity mechanism

This phenomenon could be explained by a molecular mechanism in which Cd is transported into bone cells and disturbs their functions [44, 45]. Lévesque et al. [46] reported that Cd accumulated in human osteoblasts in a dose-dependent manner and the calculated LC_{50} value of $CdCl_2$ was $22 \pm 2 \mu M$. Cd also induces apoptosis via an increase in cytochrome C release from mitochondria and caspase 3 in osteoblast cell [47-49]. Cd can disturb signal transduction in osteosarcoma cell causing a decrease of collagen synthesis [50] in addition to accelerating differentiation of new osteoclasts and activates the activity of mature osteoclasts inducing bone resorption [51].

PROPOSED Cd OSTEOToxicity MECHANISM

The mechanism to explain Cd osteotoxicity is shown in Figure 1. After Cd is absorbed into the human body, it accumulates in the kidney and bone. The direct effect of Cd on bone cells is related to the functions of osteoclasts and osteoblasts. Cd induces apoptosis of osteoblasts and decreases its activity which in turn decreases collagen synthesis and bone calcification. Cd activates the function of osteoclasts by increasing bone resorption and releasing Ca to blood circulation. The long term decreased osteoblast activity and increased osteoclast activity causes bone metabolism imbalance and decreases bone mineral density.

The indirect effect is explained by the disturbance of proximal tubules of kidney. The proximal tubule is the major site for essential

nutrient reabsorption and Vitamin D active metabolite production. After proximal tubules are destroyed by Cd, the hydroxylation of vitamin D and vitamin D active metabolite production decrease. As a result the Ca absorption from GI tract decreases causing low blood Ca and increased PTH production. The PTH enhances bone resorption to increase blood Ca which in turn causes decreased bone Ca.

Another effect of proximal tubule on bone health is that it has a function to reabsorb Ca from urine. With proximal tubular dysfunction by Cd toxicity, the reabsorption of Ca is decreased which in turn decreases blood Ca. The decreased blood Ca is the signal to enhance bone resorption. Long term bone resorption and loss of Ca to urine causes decreased bone mineral mass and eventually bone pathology such as osteopenia, osteoporosis and osteomalacia.

The clarification of Cd osteotoxicity will be advantageous for health management in Cd exposed inhabitants and prevent the severity of osteotoxicity, for example, in Itai-itai patients. These patients showed low level of serum vitamin D active metabolite and severe proximal tubular dysfunction of the indirect mechanism. The treatment with vitamin D active metabolite increased the bone density and relieved osteotoxicity severity [9, 12, 13]. A full explanation of Cd osteotoxicity mechanism is necessary as the exposure of Cd has been reported worldwide and the exposed population has increased. Other factors shown to be related to Cd

osteotoxicity are Cd exposure level, population ethnics, sex, nutrition status, children number, sunlight exposure and occupation. Therefore, further studies need to take these parameters into account to clarify the Cd osteotoxicity.

CONCLUSION

Because of its long biological half life in the human body, Cd can cause a wide range of chronic diseases as mentioned above. The bone disease is a typical characteristic of Cd exposure which is a significant health problem worldwide. It could be explained by direct and indirect mechanisms after long term of exposure. The direct mechanism is related to the disturbance of osteoblast and osteoclast functions whereas the indirect mechanism is explained by the wasting of Ca, decreased vitamin D active metabolite production and decreased Ca absorption from GI tract.

The Ca store and vitamin D levels are the key prevention factors for bone health. A sufficient body store of Ca will be advantageous to prevent bone loss by Ca wasting. An application of the proposed mechanism can be used in clinical care of high risk group who show low level of vitamin D. A vitamin D supplement will help to prevent bone loss caused by decreased vitamin D metabolism in kidney and this application has already been applied in Itai-itai patients whose serum vitamin D active metabolite was very low. Vitamin D active metabolite supplement in Itai-itai patients had been shown to reduce bone mass loss and improve osteomalacia [9]. In addition, Cd accumulation at high level enhances proximal tubular dysfunction which disturbs vitamin D metabolism and increases Ca wasting. If the exposure continues the incidence of bone disease among the inhabitants of Cd polluted area will increase. Food Cd level determination in polluted area should be an important indicator of this toxic metal trail of distribution and its entry into the food chain. The nutritional status of the population living in Cd polluted areas, especially of pregnant women, should be determined to ensure that their Fe, Zn, and Ca stored levels are enough to sustain the homeostasis effects of DMT and therefore reduce Cd absorption from GI tract.

With rapid industrial growth, the contamination of Cd is becoming a significant public health problem which implies the risk of bone diseases. A successful control of industrial Cd contamination is essential for the prevention of Cd entry into the food chain leading to Cd induced bone diseases.

ACKNOWLEDGEMENT

I would like to express my gratitude to Assistant Professor Dr. Mitra Cassely for her kind manuscript proof reading.

REFERENCES

1. Järup L, Berglund M, Elinder CG, Nordberg G, Vahter M. Health effects of cadmium exposure-a review of the literature and a risk estimate. *Scand J Work Environ Health*. 1998; 24(suppl 1): 1-52.
2. Nawrot T, Staessen J, Roels H, Munters E, Cuypers A, Richart T, et al. Cadmium exposure in the population: from health risks to strategies of prevention. *BioMetals*. 2010; 23(5): 769-82.
3. Simmons RW, Pongsakul P, Saiyasitpanich D, Klinphoklap S. Elevated Levels of Cadmium and Zinc in Paddy Soils and Elevated Levels of Cadmium in Rice Grain Downstream of a Zinc Mineralized Area in Thailand: Implications for Public Health. *Environ Geochem Health*. 2005; 27(5): 501-11.
4. Swaddiwudhipong W, Limpatanachote P, Mahasakpan P, Somyos K, Padungtod C. Cadmium-exposed population in MaeSot district, Tak province: 1. Prevalence of high urinary cadmium levels in the adults. *J Med Assoc Thai*. 2007; 90: 143-8.
5. Vahter M, Åkesson A, Lidénb C, Ceccatelli S, Berglund M. Gender differences in the disposition and toxicity of metals. *Environ Res*. 2007; 104(1): 85-95.
6. Vahter M, Berglund M, Åkesson A, Lidénb C. Metals and Women's Health. *Environ Res*. 2002; 88(3): 145-55.
7. Järup L. Cadmium overload and toxicity. *Nephrology Dialysis Transplantation*. 2002; 17(suppl 2): 35-9.
8. Järup L, Åkesson A. Current status of cadmium as an environmental health problem. *Toxicol Appl Pharmacol*. 2009; 238(3): 201-8.
9. Kasuya M, Aoshima K, Katoh T, Teranishi H, Horiguchi H, Kitagawa M, et al. Natural history of Itai-itai disease: A long-term observation on the clinical and laboratory findings in patients with Itai-itai disease; 1992. In Cook ME, Hiscock SA, Morrow H, Volpe RA. Seventh International Cadmium Conference. New Orleans: Cadmium Association; 1992. p. 180-92.
10. Kasuya M. Recent epidemiological studies on itai-itai disease as a chronic cadmium poisoning in Japan. *Water Sci Technol*. 2000; 42(7-8): 147-54.
11. Nogawa K, Kido T. Itai-Itai Disease and Health Effects of Cadmium. In: Chang LW, Magos L, Suzuki T (eds.), Florida: Toxicology of Metals. CRC. Press.; 1996. p. 353-69.
12. Nishijo M, Nagakawa H, Morikawa Y, Tabata M, Miura K, Kawano S, et al. Clinical courses and prognosis of itai-itai disease patients induced by chronic cadmium exposure. In: Collery, PH, Corbella, J, Domingo, JL, Etienne, JC, Llobet, JM (eds.), Metal Ions in Biology and Medicine. Paris: John Libbey Eurotext; 1996. p. 608-10.
13. Aoshima K, Kasuya M. Preliminary study on serum levels of 1, 25-dihydroxyvitamin D and 25-hydroxyvitamin D in cadmium-induced renal tubular

- dysfunction. *Toxicol Lett.* 1991; 57(1): 91-9.
14. Nogawa K, Tsuritani I, Kido T, Honda R, Yamada Y, Ishizaki M. Mechanism for bone disease found in inhabitants environmentally exposed to cadmium: decreased serum 1 α , 25-dihydroxyvitamin D level. *Int Arch Occup Environ Health.* 1987; 59(1): 21-30.
 15. Hayashi Y, Kobayashi E, Okubo Y, Suwazono Y, Kido T, Nogawa K. Excretion levels of urinary calcium and phosphorus among the inhabitants of Cd-polluted Kakehashi River basin of Japan. *Biol Trace Elem Res.* 2003; 91(1): 45-55.
 16. Honda R, Tawara K, Nishijo M, Nakagawa H, Tanebe K, Saito S. Cadmium exposure and trace elements in human breast milk. *Toxicology.* 2003; 186(3): 255-9.
 17. Engström A, Skerving S, Lidfeldt J, Burgaz A, Lundh T, Samsioe G, et al. Cadmium-induced bone effect is not mediated via low serum 1,25-dihydroxy vitamin D. *Environ Res.* 2009; 109(2): 188-92.
 18. Jin T, Nordberg G, Ye T, Bo M, Wang H, Zhu G, et al. Osteoporosis and renal dysfunction in a general population exposed to cadmium in China. *Environ Res.* 2004; 96(3): 353-9.
 19. Berglund M, Åkesson A, Bjellerup A, Vahter M. Metal-bone interaction. *Toxicol Lett.* 2000; 112-113: 219-25.
 20. Houillier P, Nicolet-Barousse L, Maruani G, Paillard M. What keeps serum calcium levels stable? *Joint Bone Spine.* 2003; 70(6): 407-13.
 21. Kazantzis G. Cadmium, osteoporosis and calcium metabolism. *BioMetals.* 2004; 17(5): 493-8.
 22. van Leeuwen JPTM, van den Bermd G-JCM, van Driel M, Buurman CJ, Pols HAP. 24,25-Dihydroxyvitamin D3 and bone metabolism. *Steroids.* 2001; 66(35): 375-80.
 23. Jones G, Strugnell SA, DeLuca HF. Current Understanding of the molecular actions of vitamin D. *Physiol Rev.* 1998; 78(4): 1193-231.
 24. Fazzalari NL. Bone remodeling: A review of the bone microenvironment perspective for fragility fracture (osteoporosis) of the hip. *Semin Cell Dev Biol.* 2008; 19(5): 467-72.
 25. Martin TJ, Seeman E. Bone remodelling: its local regulation and the emergence of bone fragility. *Best Practice & Research Clinical Endocrinology & Metabolism.* 2008; 22(5): 701-22.
 26. Nogawa K, Suwazono Y. Itai-itai Disease. In: Jerome, ON (ed.), *Encyclopedia of Environmental Health.* Burlington: Elsevier; 2011. p. 308-14.
 27. Chen X, Zhu G, Jin T, Lei L, Liang Y. Bone mineral density is related with previous renal dysfunction caused by cadmium exposure. *Environ Toxicol Pharmacol.* 2011; 32(1): 46-53.
 28. Aoshima K, Fan J, Cai Y, Katoh T, Teranishi H, Kasuya M. Assessment of bone metabolism in cadmium-induced renal tubular dysfunction by measurements of biochemical markers. *Toxicol Lett.* 2003; 136(3): 183-92.
 29. Nambunmee K, Honda R, Nishijo M, Swaddiwudhipong W, Nakagawa H, Ruangyuttikarn W. Bone resorption acceleration and calcium reabsorption impairment in a Thai population with high cadmium exposure. *Toxicol Mech Methods.* 2010; 20(1): 7-13.
 30. Horiguchi H, Oguma E, Sasaki S, Miyamoto K, Ikeda Y, Machida M, et al. Environmental exposure to cadmium at a level insufficient to induce renal tubular dysfunction does not affect bone density among female Japanese farmers. *Environ Res.* 2005; 97(1): 83-92.
 31. Kido T, Honda R, Tsuritani I, Ishizaki M, Yamada Y, Nakagawa H. Serum levels of bone gla-protein in inhabitants exposed to environmental cadmium. *Arch Environ Health.* 1991; 46(1): 475-81.
 32. Kido T, Honda R, Tsuritani I, Ishizaki M, Yamada Y, Nogawa K, et al. Assessment of cadmium-induced osteopenia by measurement of serum bone Gla protein, parathyroid hormone, and 1 α ,25-dihydroxyvitamin D. *J Appl Toxicol.* 1991; 11(3): 161-6.
 33. Kido T, Nogawa K, Yamada Y, Honda R, Tsuritani I, Ishizaki M, et al. Osteopenia in inhabitants with renal dysfunction induced by exposure to environmental cadmium. *Int Arch Occup Environ Health.* 1989; 61(4): 271-6.
 34. Nogawa K, Kobayashi E, Yamada Y, Honda R, Kido T, Tsuritani I, et al. Parathyroid hormone concentration in the serum of people with cadmium-induced renal damage. *Int Arch Occup Environ Health.* 1984; 54(3): 187-93.
 35. Nogawa K, Tsuritani I, Kido T, Honda R, Ishizaki M, Yamada Y. Serum vitamin D metabolites in cadmium-exposed persons with renal damage. *Int Arch Occup Environ Health.* 1990; 62(3): 189-93.
 36. Tsuritani I, Honda R, Ishizaki M, Yamada Y. Impairment of vitamin D metabolism due to environmental cadmium exposure, and possible relevance to sex-related differences in vulnerability to the bone damage. *J Toxicol Environ Health.* 1992; 37: 519-33.
 37. Wallin M, Sallsten G, Fabricius-Lagging E, Ohrn C, Lundh T, Barregard L. Kidney cadmium levels and associations with urinary calcium and bone mineral density: a cross-sectional study in Sweden. *Environ Health.* 2013; 12(1): 22.
 38. Åkesson A, Bjellerup P, Lundh T, Lidfeldt J, Nerbrand C, Samsioe G, et al. Cadmium-induced effects on bone in a population-based study of women. *Environ Health Perspect.* 2006; 114(6): 830-4.
 39. Nawrot T, Geusens P, Nulens TS, Nemery B. Occupational cadmium exposure and calcium excretion, bone density, and osteoporosis in men. *J Bone Miner Res.* 2010; 25(6): 1441-5.
 40. Tsuritani I, Honda R, Ishizaki M, Yamada Y, Aoshima K, Kasuya M. Serum bone-type alkaline phosphatase activity in women living in a cadmium-polluted area. *Toxicol Lett.* 1994; 71(3): 209-16.
 41. Omote S, Kido T, Nishijo M, Nakagawa H, Suwazono Y, Kobayashi E, et al. Urinary type I collagen cross-linked N-telopeptides in inhabitants 18 years after cessation of exposure to cadmium in Japan. *Bull Environ Contam Toxicol.* 2006; 76(2): 187-94.
 42. Schutte R, Nawrot TS, Richart T, Thijs L, Vanderschueren D, Kuznetsova T, et al. Bone resorption and environmental exposure to cadmium in women: a population study. *Environ Health Perspect.* 2008; 116(6): 777-83.

43. Bhattacharyya MH. Cadmium osteotoxicity in experimental animals: mechanisms and relationship to human exposures. *Toxicol Appl Pharmacol.* 2009; 238(3): 258-65.
44. Anke M, Latunde-Dada O, Arnhold W, Gleis M, Anke S, Hartmann E. The influence of age, sex and cadmium exposure on the ash, calcium, phosphorus, trace element and ultra trace element content in skeleton, kidneys and liver of humans; 1998. In Nogawa, K, Kurachi, M, Kasuya, M. International conference on itai-itai disease, environmental cadmium pollution and countermeasures. Kanazawa: Eiko Laboratory; 1998. p.78-86.
45. Lanocha N, Kalisinska E, Danuta Kosik-Bogacka, Budis H, Sokolowski S, Bohatyrewicz A. Comparison of concentrations of lead and cadmium in various parts of the femur head in patients after arthroplasty of the hip joint in Northwest Poland. *Biomed Environ Sci.* 2012; 25(5): 577-82.
46. Lévesque M, Martineau C, Jumarie C, Moreau R. Characterization of cadmium uptake and cytotoxicity in human osteoblast-like MG-63 cells. *Toxicol Appl Pharmacol.* 2008; 231(3): 308-17.
47. Chen L, Liu L, Huang S. Cadmium activates the mitogen-activated protein kinase (MAPK) pathway via induction of reactive oxygen species and inhibition of protein phosphatases 2A and 5. *Free Radical Biology & Medicine.* 2008; 45: 1035-44.
48. Kondoh M, Ogasawara S, Araragi S, Higashimoto M, Sato M. Cytochrome C release from mitochondria induced by cadmium. *Health Sci.* 2001; 47: 78-82.
49. Li Y, Lim S-C. Cadmium-induced apoptosis of hepatocytes is not associated with death receptor-related caspase-dependent pathways in the rat. *Environ Toxicol Pharmacol.* 2007; 24: 231-8.
50. Long GJ. Cadmium perturbs calcium homeostasis in rat osteosarcoma (ros 17/2.8) cells; a possible role for protein kinase c. *Toxicol Lett.* 1997; 91(2): 91-7.
51. Wilson AK, Cerny EA, Smith BD, Wagh A, Bhattacharyya MH. Effects of cadmium on osteoclast formation and activity in Vitro. *Toxicol Appl Pharmacol.* 1996; 140(2): 451-60.