CHAPTER III
RESULTS

3.1 Population characteristics

Seven hundred subjects from 13 villages in Mae Sot were 261 men (37.28%) and 439 women (62.71%). The average±standard deviation of age of men was 55.48±14.01 years whereas of women was 53.04±12.69 years. Body mass index was ranged between 12.88 to 32.40 kg/m^2 in men and 10.96 to 38.01 in women. The mean body mass index of both genders was less than the normal level of 25 kg/m^2. Detailed of the data included weight and height were shown in Table 2.

Farmer was the main occupation of the study subjects (n=362). Number of smoker (n=263), ex-smoker (n=203) and non-smoker (n=234) subjects were similar in approximately over 200 people but number of men smoker (n=157) were more than women (n=106) (Table 3).

Number of subjects with hypertension (n=131) was more than subjects with diabetes (n=55) and hypercholesterolemia (n=113). Details of the data in men and women were separately shown in Table 3.

Rice was a staple food of Thai population and it was proposed as a main source of cadmium (Cd) exposure in these inhabitants. Most men ate sticky rice more than women in approximately 850 g/day but 660 g/day by women. Amount of cadmium contaminated in rice was not investigated in this study, therefore, the result of this finding was discussed in Chapter IV referring to previous study.

3.2 Cadmium exposure

Blood cadmium levels among the study inhabitants (n=700) were shown in Table 4 and Figure 5 in both men (n=261) and women (n=439). Men below 39 years old had blood cadmium higher than women below the same age significantly with p <0.001 but in other age groups, blood cadmium in men and women were not significantly different. The levels of blood cadmium increased when age increased in both men and women except at the eldest people group aged over 70 years old. However, total blood cadmium in men and women were not significantly different. The significant result was the average levels of blood cadmium in the study subjects were higher than the reported cadmium levels in normal Thai population[145] indicated high cadmium exposure of the Mae Sot inhabitants.

In accordant to high levels of blood cadmium in the study subjects, the urinary cadmium in both men and women were also high (Table 5 and Figure 6). The average of urinary cadmium of the subjects were 3 times higher than accepted level of normal people at the concentration of 2 μg/g Cr reported by WHO in 1999[2]. Urinary cadmium was also increased when age increased except in the eldest people group aged over 70 years old.

3.3 Exposure to cadmium, occupation and smoking status adjusted by age

Relations between blood and urinary cadmium and occupation and smoking habit or status adjusted by age with generalized linear model are shown in Table 6 & 7. Farmers had the highest levels of blood and urinary cadmium at the concentrations of 6.08$\pm$1.13 μg/l and 7.27$\pm$1.13 μg/g Cr, respectively, which were higher than those of other occupations in both men and women. The employee and subjects with other occupations showed the lowest blood and urinary cadmium levels at the concentrations of 4.44±1.24 μg/l and 5.30±1.24 μg/g Cr, respectively. However, smoking men had blood cadmium concentrations (7.31±1.20 μg/l) but not urinary cadmium concentration (6.38±1.20 μg/g Cr) higher than women (6.39±1.26 μg/l and 7.16±1.26 μg/g Cr, respectively). All of these differences were not statistically significant. An interesting data found was smoking women apparently had lower levels of urinary cadmium than non-smokers which led us to assume that the Mae Sot women would have exposed to other source of polluted cadmium such as local rice grown in the area, not from the cigarette smoke.

3.4 Correlation of blood and urinary cadmium

Positive correlation of blood and urinary cadmium was significantly shown by scatter plot and Spearman rho correlation coefficient values of 0.669 and 0.671 in men (n=239) and women (n=441), respectively (Figure 7).

3.5 Renal dysfunction biomarkers

Concentrations of β_2-MG and NAG, the biomarkers of proximal tubular dysfunction, and serum cystain C, the biomarker of glomerular dysfunction were shown in Table 8 in comparison to blood and urinary cadmium between 700 Mae Sot inhabitants. Mean ± standard deviation of β_2-MG in men (367.96 ± 9.45 μg /g Cr) was significantly ($p<0.001$) higher than women (182.60 ± 6.05 μg /g Cr). Whereas the NAG concentrations of men and women were not significantly different. Mean ± standard deviation of serum cystatin C in men (1.17 ± 1.36 mg/l) was significantly higher than those of serum cystacin C in women (1.05 ± 1.35 mg/l) with $p<0.001$.

These results indicate that both proximal tubule and glomerular of the nephrons of Mae Sot people in average are not in normal functions even though the NAG levels are still appeared to be normal.

3.6 Cadmium exposure VS renal and bone markers

The mean and range of subject age, blood and urinary cadmium, renal dysfunction markers (urinary β_2-MG and NAG, serum octeocalcin), bone resorption markers (urinary DPD and NTx), urinary calcium and fractinal calcium excretion (FECa) in 412 subjects (men=156, women=256) whose age over 50 years were shown in Table 9 indicated high exposure to cadmium affected to renal tubular function and bone metabolism. The mean age and β_2-MG concentrations were significantly higher in men than women. Whereas, urinary calcium, DPD and NTx were significantly higher in women than men.

The concentrations of both bone resorption markers in the average urine samples were higher than the bone fracture risk cutoff values proposed by Nishizawa et al. (2004)[134] (*viz*, DPD >7.6 nmol/mmol Cr and NTx > 54.3 nmol BCE/mmol Cr). Average concentrations of urinary cadmium and NAG, serum calcium and FECa were not significantly different between men and women.

3.7 Dose-response relationship between cadmium exposure and renal and bone markers

Dose-response relationship between urinary cadmium and the renal and bone markers concentrations were investigated by grouping the subjects according to the urinary excretion of cadmium. A low level exposure group (n=20) was defined with

<2 μg Cd/g Cr; a medium exposure group (n=86 and 190) with 2-5 and 5-10 μg Cd/g Cr, respectively; and a very high exposure group (n=116) with >10 μg Cd/g Cr (Table 10 and 11).

The average concentrations of the renal and bone markers increased with rising cadmium exposure and the fractional excretion of calcium also increased, especially in women. There was also a slight decrease in serum calcium in women participants, indicative of calcium reabsorption impairment. Bone resorption acceleration was also evident, especially among subjects with high urinary cadmium excretion >10 μg Cd/g Cr.

A comparison of excreted urinary renal and bone markers in the high (>10 μg Cd/g Cr) and medium (5-10 μg Cd/g Cr) cadmium exposure groups showed NAG was significantly high in both genders. Whereas in women, urinary β_2-MG, fractional excretion of calcium and NTx were all significantly different.

These results show that high cadmium concentrations affected the renal function, accelerated bone resorption and impaired calcium reabsorption in elderly Thai women more than in men.

3.8 Gender dependent correlation between cadmium exposure, renal dysfunction and bone metabolism impairment

The correlation between cadmium exposure and urinary renal tubular dysfunction and bone metabolism impairment was analyzed and used Spearman's rho value to estimate the strength of the correlation (Table 12). Blood and urinary cadmium showed positive correlation with the bone markers. Interestingly, both bone resorption markers (DPD and NTx), were significantly correlated to the urinary cadmium concentrations in men but only NTx was highly correlated to the urinary cadmium concentrations in women. Blood cadmium was significantly correlated with DPD in men and correlated with osteocalcin, DPD and NTx in women.

A highly significant positive correlation between NTx and urinary cadmium and FECa in women was clear (Figure 8 and Figure 9) with $r = 0.231$, $p < 0.001$ and $r = 0.440$, $p < 0.001$, respectively. In addition, the bone formation markers, serum osteocalcin, was well correlated to age in both men and women but age and bone resorption markers (DPD and NTx) in both genders were not correlated (Spearman's rho value was below 0.1). The renal dysfunction markers in both men and women were positively correlated with the serum osteocalcin values. However, only the NTx in men and the DPD in women were highly correlated with the renal markers.

Urinary cadmium related well to DPD and NTx but showed no relation with serum osteocalcin. On the other hand, blood cadmium showed positively significant relation to all of bone markers. β_2-MG showed significant relation to every bone markers for both adjusting by urinary cadmium or blood cadmium. FECa was a strong explanatory variable of NTx with unstandardized coefficient 0.257 adjusted by urinary cadmium and 0.263 adjusted by blood cadmium (Table 13 and Table 14).

Unexpectedly, the DPD was inversely correlated to the urinary β_2-MG in women, whereas NTx was positively related to urinary β_2-MG, NAG and FECa independent of blood and urinary cadmium and age. Serum osteocalcin was also positively related to urinary β_2-microglobulin in both genders and only to urinary NAG in women.

3.9 Anemia prevalence among inhabitants in cadmium polluted area

Hemoglobin (Hb) in the subjects range between 3.30-18.20 g/dl (6.70-18.20 g/dl in men and 3.30-16.60 g/dl in women). Urinary cadmium of anemia subjects was significantly higher than non-anemia subjects ($p<0.05$) (Table 15). Red blood cell counts was also low in both men (4.53 ± 0.98 $10^6/\mu l$) and women (4.42 ± 0.83 $10^6/\mu l$) whereas MCV of anemic men was within normal range but in women the MCV (78.57 ± 11.29 fl) was lower than the normal values. Means values of MCH and MCHC in both anemic men and women were lower than the normal reference level whereas RDW(%) in both genders were higher than the reference level (Table 15).

Blood indices levels of anemia subjects were shown according to urinary cadmium levels in Table 16. Even though the mean level of red blood cells of the women who had anemia with urinary cadmium below 2 μg/g Cr was higher than those of other studied group, but this relation was not statistically significant ($p=0.072$). Mean of RDW of the anemic women with urinary cadmium below <2 μg/g Cr was higher than other groups (ANOVA p-value = 0.019). Moreover, the Hb, Hct, MCV, MCH and MCHC showed no relation to the urinary cadmium levels in both genders.

Correlations between Hb and Hct and age, BMI, cadmium exposure markers and renal dysfunction markers were determined by Spearman's rho analysis (Table 16). In men, Hb and Hct showed significant correlation with age, BMI, urinary cadmium, β_2-MG, NAG and cystatin C. The Hb values showed strong negative correlation to age ($r=-0.498$, $p<0.001$). In women, Hb and Hct showed significant correlation with age, BMI, blood cadmium, β_2-MG, NAG and cystatin C. In addition, Hb values showed negative correlation to urinary cadmium in both genders and this correlation was statistically significant in only men (Figure 10). Age was also showed significant relation to anemia prevalence in men ($p<0.001$) and borderline significant in women ($p=0.055$)(Table 17).

Table 18 and 19 showed the relation between renal dysfunction severity and anemia. Even though urinary cadmium showed no relation to anemia prevalence (Chi-square p-value = 0.228) but anemia prevalence in people with urinary cadmium >10 μg/g Cr was higher than others (30.65%). Anemia prevalence in the subjects with β_2-MG > 1,000 μg/g Cr was 37.97% which was significantly higher than those of β_2-MG <400 and 400-1,000 μg/g Cr. The prevalence of anemia in the subjects with NAG >8 unit/g Cr (41.38%) was significantly higher than others. Cystatin C also showed significant relation to anemia prevalence, the prevalence of anemia in the subjects with cystatin C >1.4 mg/l was 50.91% which was higher than other subjects.

In women, urinary cadmium showed significant relation to anemia prevalence with Chi-square p-value = 0.034 and the prevalence of anemia in urinary cadmium >10 μg/g Cr (32.50%) was higher than other subjects (Table 19). β_2-MG, NAG and cystatin C showed substantial significant relation to anemia prevalence ($p<0.001$). The prevalence of anemia in β_2-MG >1,000 μg/g Cr, NAG >8 Unit/g Cr and cystatin C >1.4 mg/l was 42.86%, 33.33% and 41.67%, respectively, which was higher than other groups significantly ($p<0.001$).

The relations between an increase prevalence of anemia and age, urinary cadmium, renal dysfunction markers were verified by logistic regression model (Table 20 and 21). Age was a significant related to anemia prevalence in men (expected odds p-value<0.001) whereas urinary cadmium and renal dysfunction markers were not. The expected odds ratio of women with β_2-MG >1,000 was 2.77 with statistically significance ($p<0.001$). The odds ratios of women with NAG>8 unit/g Cr and cystatin C >1.4 mg/l were 3.28 and 2.86, respectively ($p<0.05$). The Nagelkerke R square in model included age, log urinary cadmium and β_2-MG of women were higher than the model included NAG or cystatin C (R^2= 0.07 vs 0.05 and 0.05, respectively).

3.10 Effect of cadmium on hFOB 1.19 mRNA expression

Morphology of the human fetal osteoblast like cells (hFOB cells) after treated with $CdCl_2$ concentrations of 0.31-2.50 μM was similar to the control cells showing their cell's adherence without round shape cells or cell scrum. At $CdCl_2$ concentration of 10.00 μM, there were round shape cells shown with detached cells. Cell detachment was dominantly shown at the $CdCl_2$ concentration of 20 μM and healthy cell population in the culture media was diminished after treatment with $CdCl_2$ concentration of 40 μM (Figure 11).

LC50 of $CdCl_2$ was calculated (Figure 12) and shown at the concentration of 23.17 μM whereas LC20 was 7.72 μM. Figure 13 shows high correlation between the two cytotoxicity assays, MTT and trypan blue exclusion asssays with correlation coefficient of 0.817. Therefore, the LC20 obtained from the MTT assay was chosen to use for the gene expression experiments.

The expression of osteocalcin (OC), bone alkaline phosphatase (ALP), type I collagen (Col1A1), RANKL and OPG genes of the hFOB 1.19 cells after treatment with $CdCl_2$ solution were investigated and found that only the OC, Col1A1 and OPG mRNA were detected in every determined condition but not the ALP and RANKL mRNA using dexamethasone 1 x 10^{-6} M as positive control (Figure 14).

The expression of Col1A1 was not related to concentration of $CdCl_2$ and at the concentration of 7.72 μM the mRNA level was decreased to 0.509 fold of the control (Figure 14). Even though there was no relation between $CdCl_2$ concentrations and the genes expression, the $CdCl_2$ at the concentrations of 1.83, 3.86 and 7.72 μM showed an increased of the OPG gene expression more than the control and $CdCl_2$ at the concentration of 7.72 μM showed the highest OPG gene expression in all experimental conditions (4.344 ± 0.786 folds compared to the control). However, the results need more experiments to clarify assumption that cadmium has a direct effect to the osteoblast causes several genes expression as it's mechanism of toxicity.

Table 2 Characteristics of 700 surveryed inhabitants living in Mae Sot district, Tak province, a cadmium polluted area

	Men (n=261)			Women (n=439)			Total (n=700)		
	Mean*	**S.D.**	**Min - Max**	**Mean**	**S.D.**	**Min - Max**	**Mean**	**S.D.**	**Min - Max**
Age (years)	55.48	14.01	23 - 89	53.04	12.69	21 - 87	53.95	13.24	21 - 89
Weight (kg)	55.46	13.62	29 - 163	51.40	10.52	25 - 89	52.91	11.93	25 - 163
Height(cm)	159.85	11.45	46 - 180	151.08	5.07	137 - 166	154.35	9.10	46 - 180
BMI (kg/m^2)	21.07	3.13	12.88 - 32.40	22.51	4.19	10.96 - 38.01	21.97	3.89	10.96 - 38.01

*Arithmetic mean; S.D.=standard deviation, n=number of subjects; Min=minimum; Max=maximum

Table 3 Number of the study subjects classified by gender, occupation, smoking status and underlying diseases

Characteristics	Men		Women		Total	
	N	(%)	N	(%)	N	(%)
Occupation						
Unemployed	60	(22.99)	152	(34.62)	212	(30.29)
Farmer	162	(62.07)	200	(45.56)	362	(51.71)
Employee and others	39	(14.94)	87	(19.82)	126	(18.00)
Smoking status						
Non-smoker	18	(6.90)	216	(49.20)	234	(33.43)
Ex-smoker	86	(32.95)	117	(26.65)	203	(29.00)
Smoker	157	(60.15)	106	(24.15)	263	(37.57)
Underlying diseases						
Diabetes	18	(6.90)	37	(8.43)	55	(7.86)
Hypertension	50	(19.16)	81	(18.45)	131	(18.71)
Hypercholesterolemia	28	(10.73)	85	(19.36)	113	(16.14)
Total	**261**	**(100.00)**	**439**	**(100.00)**	**700**	**(100.00)**

Unemployed = retired subjects aged > 60 years; employee and others = labors, government officers, skillful workers and merchants

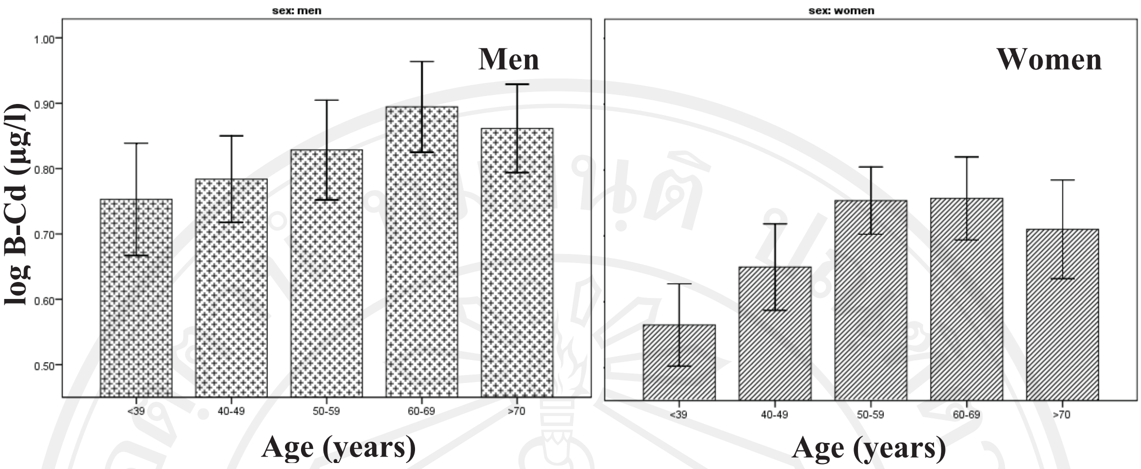

Figure 5 Histograms of blood cadmium (B-Cd) concentrations distribution according to age groups in men (left panel) and women (right panel) who live in Mae Sot, the cadmium polluted area.

Table 4 Mean ± standard deviation of blood cadmium (B-Cd) distribution according to age groups in both men and women who live in Mae Sot, the cadmium polluted area.

Age (years)	Men				Women				
	N	B-Cd (µg/l) Mean	B-Cd (µg/l) S.D.	ANOVA p-value	N	B-Cd (µg/l) Mean	B-Cd (µg/l) S.D.		ANOVA p-value
<39	30	5.55	1.71	0.053	70	3.67	1.83		<0.001
40-49	72	6.08	1.91		109	4.50	2.22		
50-59	52	6.74	1.88		118	5.69	1.92	***	
60-69	54	7.84	1.80		95	5.65	2.04	***	
>70	53	7.27	1.76		47	5.13	1.80	*	
Total	261	6.71	1.84		439	4.94	2.02		

* p<0.05 compared to the group of subjects <39 years
***p<0.001 compared to the group of subjects <39 years

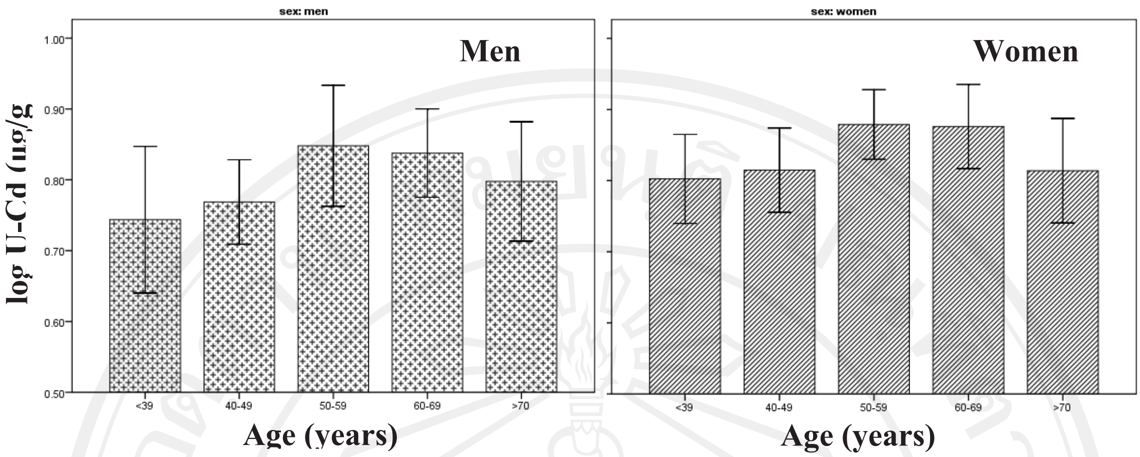

Figure 6 Histograms of urinary cadmium (U-Cd) concentrations distribution according to age groups in men (left panel) and women (right panel) who live in Mae Sot, the cadmium polluted area.

Table 5 Mean ± standard deviation of urinary cadmium (U-Cd) distribution according to age groups in both men and women who live in Mae Sot, the cadmium polluted area.

Age (years)	Men				Women			
	N	U-Cd (μg/g Cr)		ANOVA	N	U-Cd (μg/g Cr)		ANOVA
		Mean	S.D.	p-value		Mean	S.D.	p-value
<39	30	5.62	1.93	0.344	70	6.35	1.83	0.182
40-49	72	5.87	1.79		109	6.54	2.07	
50-59	52	7.05	2.03		118	7.61	1.86	
60-69	54	6.88	1.69		95	7.48	1.96	
>70	53	6.28	2.02		47	6.49	1.80	
Total	261	6.35	1.88		439	6.97	1.93	

Table 6 Relation between **blood cadmium** (B-Cd) of the study subjects and three types of classified occupations and smoking status adjusted by age

	B-Cd (µg/l) Men				**B-Cd (µg/l) Women**				**B-Cd (µg/l) Total**			
	N	Mean	S.E.		N	Mean	S.E.		N	Mean	S.E.	
Occupation group												
Farmer	162	6.98	1.20	NS	200	5.39	1.18	NS	362	6.08	1.13	NS
Unemployed	60	6.78	1.45		152	5.06	1.23		212	5.40	1.20	
Employee and other works	39	5.61	1.50		87	3.89	1.29		126	4.44	1.24	
Smoking status												
Non-smoker	18	5.24	1.73	NS	216	4.66	1.19	NS	234	4.68	1.17	NS
Ex-smoker	86	6.04	1.29		117	4.37	1.26		203	5.04	1.18	
Smoker	157	7.31	1.20		106	6.39	1.26		263	6.92	1.15	

- Adjusted mean age of men was 55.48, women was 53.04 and total subject was 53.95 years calculated by generalized linear model
- Mean comparison was analyzed by comparing the means according to occupation and smoking status using Wald chi-square.
- NS = not significant, S.E.= standard error

Table 7 Relation between **urinary cadmium** (U-Cd) of the study subjects and three types of classified occupations and smoking status adjusted by age

	U-Cd (µg/g Cr) Men				U-Cd (µg/g Cr) Women				U-Cd (µg/g Cr) Total			
	N	Mean	S.E.		N	Mean	S.E.		N	Mean	S.E.	
Occupation group												
Farmer	162	6.72	1.20	NS	200	7.84	1.18	NS	362	7.27	1.13	NS
Unemployed	60	5.80	1.45		152	7.04	1.23		212	6.79	1.20	
Employee and other works	39	5.74	1.50		87	5.22	1.29		126	5.30	1.24	
Smoking status												
Non-smoker	18	5.90	1.73	NS	216	7.39	1.19	NS	234	7.25	1.17	NS
Ex-smoker	86	6.39	1.29		117	6.12	1.26		203	6.23	1.18	
Smoker	157	6.38	1.20		106	7.16	1.26		263	6.69	1.15	

- Adjusted mean age of men was 55.48, women was 53.04 and total subject was 53.95 years calculated by generalized linear model
- Mean comparison was analyzed by comparing the means according to occupation and smoking status using Wald chi-square.
- NS = not significant, S.E.= standard error

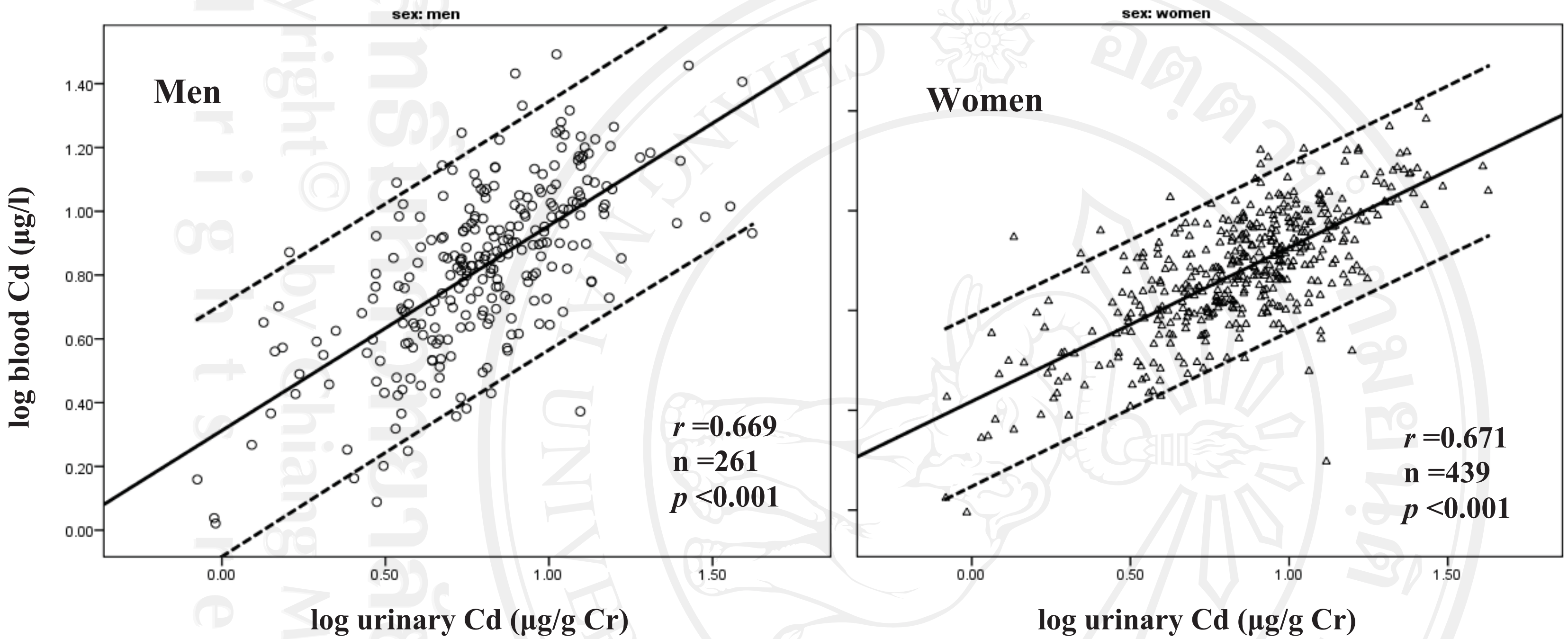

Figure 7 Correlation between blood and urinary cadmium (Cd) in men (left panel) and women (right panel) who live in cadmium polluted area, broken line represents 95% confidence interval of the correlation, *r* = Spearman 'rho correlation coefficient

Table 8 Comparison of age, body mass index, blood cadmium (B-Cd), urinary cadmium (U-Cd) and renal dysfunction markers between men and women of all study subjects living in cadmium polluted area, Mae Sot district, Tak province

	Men (n=261)			Women (n=439)			Total (n=700)			t-test
	Mean	S.D.	Min - Max	Mean	S.D.	Min - Max	Mean	S.D.	Min - Max	p-value
Age(years) [a]	55.48	14.01	23 - 89	53.04	12.69	21 - 87	53.95	13.24	21 - 89	0.022
BMI	21.07	3.13	12.88 - 32.40	22.51	4.19	10.96 - 38.01	21.97	3.89	10.96 - 38.01	<0.001
B-Cd (µg/l) [b]	6.71	1.84	1.05 - 30.90	4.94	2.02	0.31 - 33.11	5.54	1.99	0.31 - 33.11	<0.001
U-Cd (µg/g Cr) [b]	6.35	1.88	0.83 - 41.69	6.97	1.93	0.83 - 42.66	6.73	1.91	0.83 - 42.66	0.064
β_2-MG (µg/g Cr) [b]	367.96	9.45	6.46 - 74,131.02	182.60	6.05	5.50 - 107,151.93	237.14	7.43	5.50 - 107,151.93	<0.001
NAG (Unit/g Cr) [b]	5.30	1.88	0.51 - 48.98	5.66	1.85	0.52 - 245.47	5.52	1.86	0.51 - 245.47	0.167
Cystatin C (mg/l) [a]	1.17	1.36	0.63 - 4.47	1.05	1.35	0.60 - 3.89	1.09	1.36	0.60 - 4.47	<0.001

B-Cd = blood cadmium, U-Cd = urinary cadmium, Cr = creatinine, β_2-MG = urinary β_2-microglobulin, NAG = urinary N-acetyl-β-D-glucosaminidase, Cystatin C = serum cystatin C

[a] arithmetic value; [b] geometric value

Mean comparison between gender was analyzed by independent sample t-test

Table 9 Comparisons of age, body mass index, urinary cadmium, blood cadmium, renal and bone markers between men and women aged 50 years and over, living in cadmium polluted area, Mae Sot district, Tak province

	Men (N = 156)			**Women (N = 256)**			*p*-value	Reported normal level[a]
	Mean	(S.D.)	Min - Max	Mean	(S.D.)	Min - Max		
Age (year)	64.40	(9.31)	50 - 86	61.64	(8.36)	50 - 87	0.003	
BMI (kg/m^2)	20.31	(2.94)	12.88 - 30.44	21.54	(4.24)	10.96 - 32.89	0.001	25
B-Cd (µg/l)	7.20	(1.80)	1.44 – 28.65	5.54	(1.94)	0.80 - 33.12	<0.001	0.98 (0.45-2.11)[145]
U-Cd (µg/g Cr)	6.71	(1.92)	0.84 - 41.82	7.32	(1.89)	1.19 - 42.41	0.181	0.87(0.251.98)[145]
β_2-MG (µg/g Cr)	854.75	(10.38)	12.50 - 74,968.94	232.56	(6.49)	6.80 - 107,883.21	<0.001	156 $\pm$ 3.10[146]
NAG (Unit/g Cr)	6.23	(1.77)	0.51 - 48.88	6.39	(1.81)	0.53 - 35.52	0.674	4.40 $\pm$ 1.90[146]
OC (ng/ml)	4.91	(1.85)	0.50 - 18.80	5.63	(1.94)	0.50 - 29.20	0.036	4.30-9.20[36]
DPD (nmol/mmol Cr)	5.22	(1.39)	2.03 - 14.67	8.30	(1.44)	3.13 - 38.40	<0.001	2.80-7.60[134]
NTx (nmol BCE/mmol Cr)	43.50	(1.80)	10.40 - 166.50	65.16	(1.77)	11.30 - 323.70	<0.001	9.30-54.30[134]
S-Ca (mg%)	9.38	(1.06)	8.00 - 11.90	9.50	(1.07)	5.50 - 11.90	0.051	9.40 $\pm$ 0.10[106]
U-Ca (mg/g Cr)	64.60	(2.66)	3.80 - 466.67	83.91	(2.80)	3.98 - 2,000.00	0.011	185.00 $\pm$ 1.90[63]
FECa (%)	0.84	(2.43)	0.07 - 7.71	0.82	(2.59)	0.05 - 32.00	0.825	0.30 $\pm$ 5.20[36]

B-Cd = blood cadmium, U-Cd = urinary cadmium, BMI = body mass index, β_2-MG = urinary β_2-microglobulin, NAG = urinary N-acetyl-β-D-glucosaminidase, OC = serum osteocalcin, DPD= urinary deoxypyridinoline, NTx = urinary type I collagen crosslinked N-telopeptide, BCE = bone collagen equivalent, S-Ca = serum calcium, U-Ca = urinary calcium, FECa = fractional excretion of calcium

Table 10 Dose-response analysis of the concentrations of renal markers (β_2-MG and NAG) and bone markers (OC, DPD and NTx) to four levels of cadmium in **156 men** aged ≥ 50 years.

U-Cd (µg/g Cr)	<2		2-5		5-10		>10		ANOVA
	Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	***p-value***
Men	N=8		N=34		N=71		N=43		
Age (years)	65.63	13.46	66.56	8.32	63.16	9.32	64.51	9.15	0.358
B-Cd (µg/l)	3.19	1.50	4.02	1.52	7.63	1.55	12.08	1.40	<0.001
U-Cd (µg/g Cr)	1.43	1.28	3.63	1.26	6.85	1.21	14.06	1.43	<0.001
β_2-MG (µg/g Cr)	202.72	7.23	838.49	13.55	718.62	9.39	1510.08	9.52	0.112
NAG (Unit/g Cr)	4.00	2.52	5.62	1.79	5.96	1.63	7.88	1.70 ‡	0.003
S-Ca (mg%)	9.41	1.06	9.40	1.05	9.37	1.06	9.38	1.07	0.991
U-Ca (mg/g Cr)	40.07	3.20	68.17	2.65	65.90	2.82	65.48	2.34	0.567
FECa (%)	0.60	2.49	0.89	2.52	0.85	2.63	0.83	2.08	0.716
Serum OC (ng/ml)	4.75	1.61	4.85	1.83	4.77	1.82	5.22	1.97	0.897
DPD (nmol/mmol Cr)	4.51	1.79	4.84	1.35	5.12	1.33	5.90	1.39 †	0.021
NTx (nmol BCE/mmol Cr)	28.75	1.94	40.18	1.93	43.99	1.73	49.12	1.75	0.090

Mean comparison was evaluated using Dunnett's T3 method.
*: *$p<0.05$*, **: *$p<0.001$*, compared with the group of U-Cd <2 µg/g Cr , †: *$p<0.05$*, compared with the group of U-Cd 2-5 µg/g Cr
‡: p<0.05, ‡‡: p<0.01, compared with the group of U-Cd 5-10 µg/g Cr

Table 11 Dose-response analysis of the concentrations of renal markers (β_2-MG and NAG) and bone markers (OC, DPD and NTx) to four levels of cadmium in 256 **women** aged ≥ 50 years.

U-Cd (µg/g Cr)	<2		2-5		5-10		>10		ANOVA
	Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	***p-value***
Women	N=12		N=52		N=119		N=73		
Age (years)	62.83	9.06	62.23	9.11	61.53	8.05	61.21	8.33	0.870
B-Cd (µg/l)	1.71	1.92	3.56	1.61	5.43	1.62	9.55	1.70	<0.001
U-Cd (µg/g Cr)	1.62	1.18	3.71	1.25	7.37	1.21	15.08	1.38	<0.001
β_2-MG (µg/g Cr)	65.48	2.86	221.77	6.61 *	188.54	5.63 *	417.25	7.58 **,‡	0.002
NAG (Unit/g Cr)	4.00	2.19	5.94	1.87	6.06	1.76	7.90	1.69 ‡‡	<0.001
S-Ca (mg%)	9.61	1.05	9.63	1.06	9.48	1.05	9.41	1.09	0.247
U-Ca (mg/g Cr)	88.53	1.93	65.77	3.37	78.61	2.73	110.03	2.53	0.036
FECa (%)	0.79	1.83	0.69	2.94	0.75	2.51	1.10	2.48 ‡	0.018
OC (ng/ml)	5.10	1.59	5.46	2.04	5.33	1.94	6.40	1.90	0.266
DPD (nmol/mmol Cr)	7.94	1.43	8.00	1.49	8.04	1.42	9.06	1.44	0.124
NTx (nmol BCE/mmol Cr)	55.59	1.63	55.12	1.95	64.12	1.66	77.37	1.75 †	0.006

Mean comparison was evaluated using Dunnett's T3 method.
*: *$p<0.05$*, **: *$p<0.001$*, compared with the group of U-Cd <2 µg/g Cr , †: *$p<0.05$*, compared with the group of U-Cd 2-5 µg/g Cr
‡: $p<0.05$, ‡‡: $p<0.01$, compared with the group of U-Cd 5-10 µg/g Cr

Table 12 Gender dependent correlations between urinary cadmium, bone markers (rows) and renal tubular dysfunction markers (columns) in the study subjects aged ≥ 50 years

Variables	Age	B-Cd	U-Cd	β_2-MG	NAG	FECa
Men	*r*	*r*	*r*	*r*	*r*	*r*
OC	0.216**	0.144	0.123	0.260***	0.288***	0.062
DPD	0.044	0.209 **	0.225**	0.001	0.145	0.046
NTx	-0.067	0.140	0.170*	0.337***	0.213**	0.398***
Women						
OC	0.209***	0.193 **	0.148*	0.243***	0.276***	0.146*
DPD	0.012	0.128 *	0.120	-0.192**	0.175**	0.129*
NTx	-0.026	0.197**	0.231***	0.142*	0.078	0.440***

Correlation coefficients were determined by Spearman's rho analysis.
Spearman's rho (*r*)-values were used to estimate the probability of a chance correlation
* $p<0.05$, ** $p<0.01$, *** $p<0.001$

B-Cd = blood cadmium, U-Cd = urinary cadmium, β_2-MG = urinary β_2-microglobulin, NAG = urinary N-acetyl-β-D-glucosaminidase, OC = serum osteocalcin, DPD= urinary deoxypyridinoline, NTx = urinary type I collagen crosslinked N-telopeptide, FECa = fractional excretion of calcium

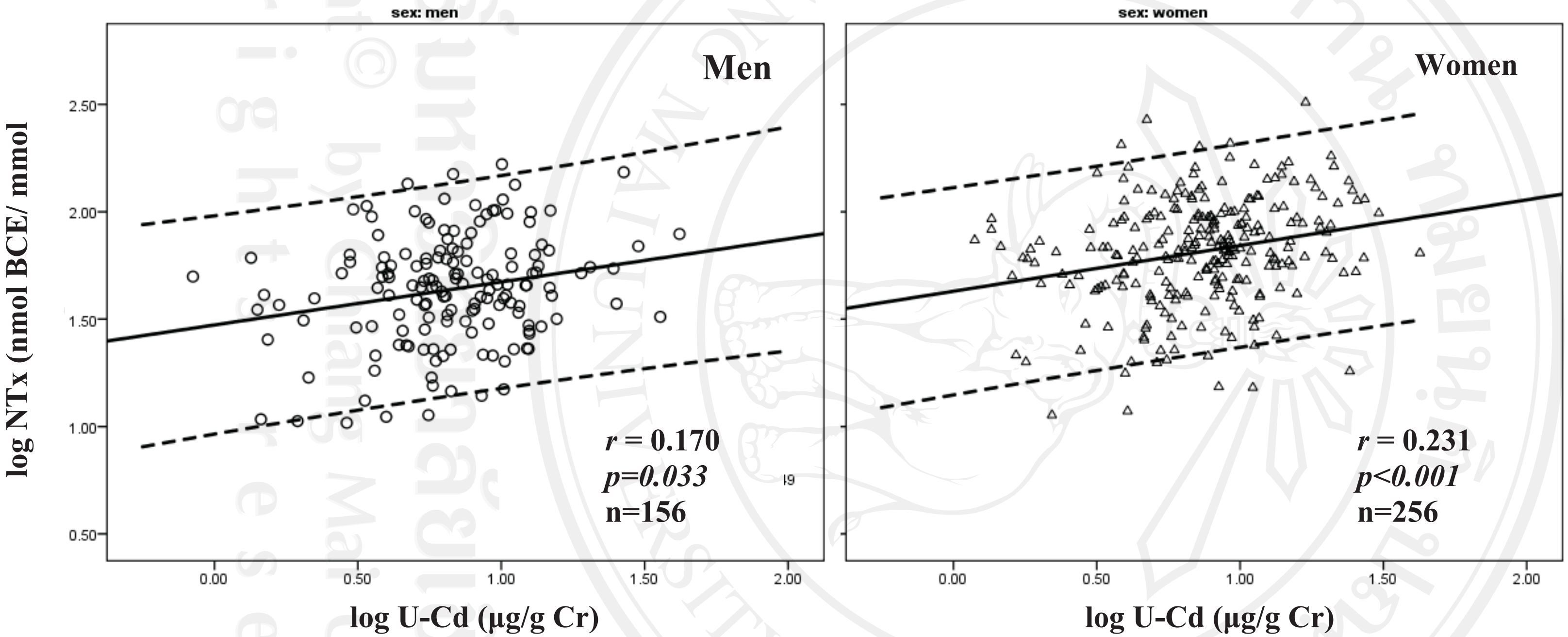

Figure 8 The relationship between bone resorption (NTx) and urinary cadmium (U-Cd) among men and women whose age ≥ 50 years from cadmium polluted area.

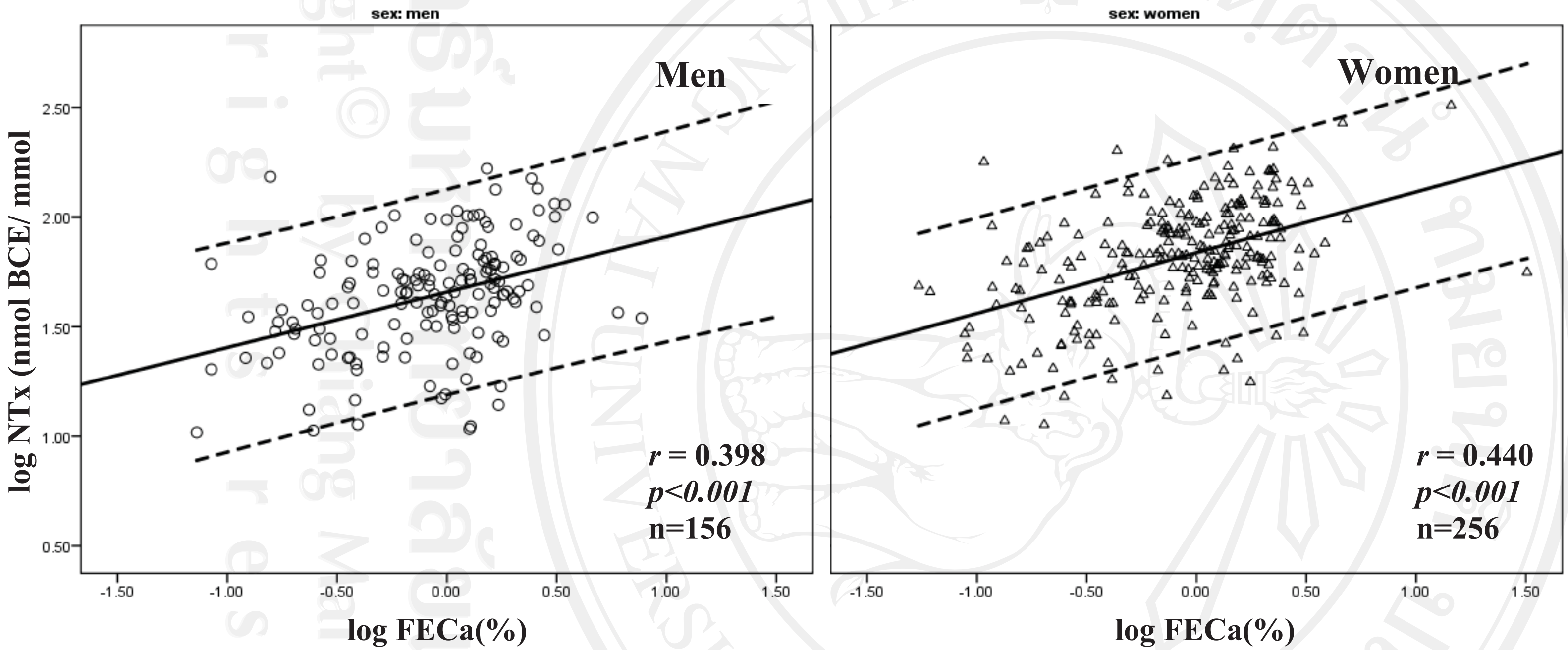

Figure 9 The relationship between bone resorption (NTx) and impaired calcium reabsorption capacity (FECa(%) among men and women whose age $\geq$ 50 years from cadmium polluted area.

Table 13 Multivariate regression analyses of age, sex, **blood cadmium** (B-Cd) and renal tubular dysfunction markers on bone markers among cadmium polluted area inhabitants aged ≥ 50 years

	OC			DPD			NTx		
	B	**S.E.**	**t**	**B**	**S.E.**	**t**	**B**	**S.E.**	**t**
Age	0.006	0.002	3.726***	0.001	0.001	1.334	-0.003	0.001	-1.924
Sex[a]	0.122	0.029	4.281***	0.199	0.016	12.243***	0.217	0.026	8.392***
B-Cd	0.110	0.048	2.272*	0.079	0.028	2.867**	0.107	0.044	2.430*
β_2-MG	0.060	0.015	3.878***	-0.025	0.009	-2.854**	0.066	0.014	4.701***
Age	0.005	0.002	3.532***	0.000	0.001	-0.486	-0.002	0.001	-1.602
Sex[a]	0.085	0.028	3.042**	0.203	0.016	12.890***	0.181	0.026	7.046***
B-Cd	0.108	0.048	2.236*	0.041	0.027	1.514	0.121	0.044	2.721**
NAG	0.240	0.054	4.417***	0.124	0.031	4.019***	0.166	0.050	3.321***
Age	0.008	0.002	5.073***	0.001	0.001	0.839	0.001	0.001	0.393
Sex[a]	0.098	0.028	3.473***	0.210	0.016	13.183***	0.192	0.023	8.202***
B-Cd	0.139	0.048	2.888**	0.058	0.027	2.133*	0.115	0.040	2.858**
FECa	0.075	0.033	2.251*	0.034	0.019	1.823	0.263	0.028	9.495***

[a] = 0 for men and 1 for women, B = unstandardized regression coefficient, S.E. = standard error, t = t-score,
* p<0.05, ** p<0.01, *** p<0.001

Table 14 Multivariate regression analyses of age, sex, **urinary cadmium** (U-Cd) and renal tubular dysfunction markers on bone markers among cadmium polluted area inhabitants aged $\geq$ 50 years

	OC			DPD			NTx		
	B	**S.E.**	**t**	**B**	**S.E.**	**t**	**B**	**S.E.**	**t**
Age	0.006	0.002	3.707***	0.001	0.001	1.653	-0.002	0.001	-1.617
Sex[a]	0.109	0.029	3.821***	0.186	0.016	11.547***	0.198	0.026	7.738***
U-Cd	0.061	0.048	1.258	0.109	0.027	4.019***	0.166	0.043	3.827***
β_2-MG	0.063	0.016	4.054***	-0.027	0.009	-3.140**	0.062	0.014	4.429***
Age	0.005	0.002	3.447***	0.000	0.001	-0.239	-0.002	0.001	-1.176
Sex[a]	0.071	0.027	2.591**	0.197	0.015	12.806***	0.163	0.025	6.532***
U-Cd	0.032	0.050	0.648	0.060	0.028	2.134*	0.170	0.045	3.748***
NAG	0.252	0.056	4.457***	0.111	0.032	3.498***	0.131	0.051	2.554*
Age	0.008	0.002	5.148***	0.001	0.001	1.019	0.001	0.001	0.624
Sex[a]	0.079	0.028	2.839**	0.201	0.016	12.891***	0.174	0.023	7.621***
U-Cd	0.090	0.048	1.864	0.086	0.027	3.173**	0.163	0.040	4.112***
FECa	0.076	0.034	2.272*	0.031	0.019	1.647	0.257	0.027	9.340***

[a] = 0 for men and 1 for women, B = unstandardized regression coefficient, S.E. = standard error, t = t-score,
* p<0.05, ** p<0.01, *** p<0.001

Table 15 Comparison of blood and urinary cadmium (B-Cd and U-Cd) concentrations to blood indices in the study subjects

	Men					Women					Total				
	Anemia (n=60)		Non-anemia (n=201)		t-test p-value	Anemia (n=102)		Non-anemia (n=337)		t-test p-value	Anemia (n=162)		Non-anemia (n=538)		t-test p-value
	Mean	S.D.	Mean	S.D.		Mean	S.D.	Mean	S.D.		Mean	S.D.	Mean	S.D.	
Blood Cd (µg/l)	6.86	1.84	6.67	1.84	0.751	4.65	2.00	5.03	2.03	0.318	5.37	1.99	5.59	1.99	0.511
U-Cd (µg/g Cr)	7.33	2.06	6.08	1.82	0.071	7.63	1.98	6.78	1.91	0.114	7.52	2.01	6.51	1.88	0.013
Hb (g/dl)	11.31	1.35	14.77	1.09	<0.001	10.87	1.27	13.43	0.93	<0.001	11.03	1.31	13.93	1.18	<0.001
Hct (%)	35.64	3.97	44.92	3.27	<0.001	34.02	3.83	41.05	2.81	<0.001	34.62	3.95	42.50	3.53	<0.001
RBC (10^6 cells/µl)	4.53	0.98	5.01	0.61	<0.001	4.42	0.83	4.69	0.50	0.003	4.46	0.89	4.81	0.57	<0.001
MCV (fl)	81.10	13.23	90.56	8.32	<0.001	78.57	11.29	88.16	7.43	<0.001	79.51	12.06	89.05	7.85	<0.001
MCH (pg)	25.89	5.23	29.80	3.09	<0.001	25.22	4.40	28.89	2.85	<0.001	25.47	4.72	29.23	2.97	<0.001
MCHC (g/dl)	31.72	1.64	32.87	0.83	<0.001	31.96	1.20	32.73	0.79	<0.001	31.87	1.38	32.78	0.81	<0.001
RDW (%)	15.73	3.85	13.19	0.98	<0.001	15.07	3.61	13.12	0.94	<0.001	15.31	3.70	13.15	0.95	<0.001

Anemia: classified with hemoglobin level <13 mg% in men and <12 mg% in women[147].
RBC= Red Blood Cell Count (Reference level: 4.5-6.0 10^6 cells/µl)
MCV=Mean Corpuscular Volume(Reference level: 80.0-97.0 fl)
MCH=Mean Corpuscular Hemoglobin(Reference level: 27.0-31.0 pg)
MCHC=Mean Corpuscular Hemoglobin Concentration(Reference level: 32.0-36.0 g/dl)
RDW=Red Blood Cell Distribution Width(Reference level: 11.5-14.5 %.

Table 16 Mean and standard deviation of blood indices of the **<u>anemic subjects</u>,** both genders, in four groups of the subjects classified by concentrations of urinary cadmium (U-Cd) from <2 to > 10 µg/g Cr

U-Cd (µg/g Cr)	<2		2-5		5-10		>10		Total		ANOVA
	Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**p-value**
Men	N=1		N=17		N=23		N=19		N=60		
RBC (10^6 cells/µl)	4.21	-	4.50	1.01	4.73	1.05	4.33	0.88	4.53	0.98	0.611
Hb (g/dl)	11.20	-	11.01	1.61	11.63	1.07	11.19	1.42	11.31	1.35	0.518
Hct (%)	35.20	-	34.78	4.23	36.54	4.02	35.33	3.79	35.64	3.97	0.566
MCV (fl)	83.50	-	80.17	16.45	79.70	12.19	83.52	11.90	81.10	13.23	0.807
MCH (pg)	26.50	-	25.57	6.56	25.58	4.91	26.52	4.61	25.89	5.23	0.937
MCHC (g/dl)	31.80	-	31.55	1.88	31.91	1.41	31.64	1.76	31.72	1.64	0.911
RDW (%)	14.20	-	16.29	4.30	14.95	2.61	16.25	4.72	15.73	3.85	0.624
Women	N=5		N=21		N=37		N=39		N=102		
RBC (10^6 cells/µl)	5.20	1.03	4.31	0.72	4.55	0.74	4.27	0.90	4.42	0.83	0.072
Hb (g/dl)	10.78	1.41	10.99	0.83	11.12	0.76	10.59	1.73	10.87	1.27	0.318
Hct (%)	34.70	4.05	34.29	2.39	34.88	2.38	32.96	5.19	34.02	3.83	0.165
MCV (fl)	68.36	13.36	81.03	10.45	78.29	11.06	78.82	11.37	78.57	11.29	0.162
MCH (pg)	21.30	4.54	26.06	4.04	25.04	4.32	25.44	4.53	25.22	4.40	0.181
MCHC (g/dl)	31.06	0.67	32.04	1.14	31.86	1.15	32.12	1.31	31.96	1.20	0.282
RDW (%)	19.86	9.20	15.28	4.55	14.68	2.18	14.71	2.66	15.07	3.61	0.019

Anemia: classified with hemoglobin level <13 mg/dl in men and <12 mg/dl in women[147].
Mean comparison was evaluated using Dunnett's T3 method

Table 17 Correlation between hemoglobin (Hb) & hematocrit (Hct), and cadmium concentrations in blood and urine, renal markers, age and body mass index (BMI) in all of the study subjects living in the cadmium polluted area

	Men (n=261)		Women (n=439)		Total (n=700)	
	Hb	Hct	Hb	Hct	Hb	Hct
Age(year)	-0.498***	-0.481***	-0.193***	-0.195***	-0.264***	-0.264***
BMI(km/m^2)	0.323***	0.303***	0.102*	0.108*	0.100**	0.093*
B-Cd (μg/l)	-0.026	-0.019	0.106*	0.135*	0.136***	0.157***
U-Cd (μg/g Cr)	-0.168**	-0.18**	-0.085	-0.076	-0.132***	-0.128***
β_2-MG (μg/g Cr)	-0.437***	-0.442***	-0.151**	-0.163***	-0.204***	-0.210***
NAG (Unt/g Cr)	-0.387***	-0.412***	-0.191***	-0.201***	-0.279***	-0.290***
Cystatin C (mg/l)	-0.477***	-0.473***	-0.198***	-0.195***	-0.209***	-0.210***

The correlations were expressed as Spearman rho coefficient values
*p<0.05, **p<0.01, ***p<0.001

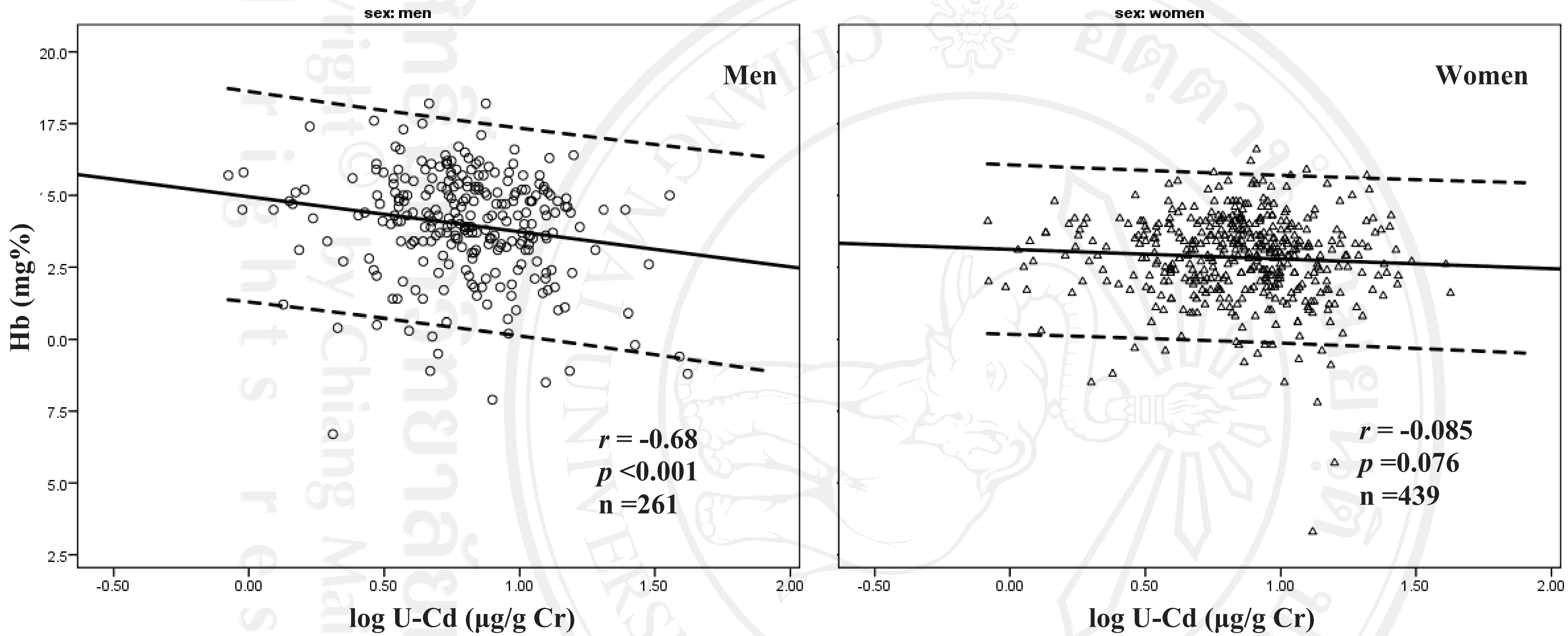

Figure 10 Correlation between urinary cadmium (U-Cd) and hemoglobin (Hb) in men (left panel) and women (right panel) of 700 Mae Sot inhabitants, the broken line represents 95% confidence interval of the correlation, r = Spearman'rho correlation coefficient.

Table 18 Relation between anemia prevalence in 5 age groups among the study subjects

Age (years)	Anemia		Non-anemia		Total		Chi-square
	N	(%)	N	(%)	N	(%)	p-value
Men							
<39	4	(13.33)	26	(86.67)	30	(100)	<0.001
40-49	4	(5.56)	68	(94.44)	72	(100)	
50-59	11	(21.15)	41	(78.85)	52	(100)	
60-69	12	(22.22)	42	(77.78)	54	(100)	
>70	29	(54.72)	24	(45.28)	53	(100)	
Total	60	(22.99)	201	(77.01)	261	(100)	
Women							
<39	16	(22.86)	54	(77.14)	70	(100)	0.055
40-49	16	(14.68)	93	(85.32)	109	(100)	
50-59	29	(24.58)	89	(75.42)	118	(100)	
60-69	24	(25.26)	71	(74.74)	95	(100)	
>70	17	(36.17)	30	(63.83)	47	(100)	
Total	102	(23.23)	337	(76.77)	439	(100)	

Anemia = Hb<13 g/dl in men and <12 g/dl in women

Table 19 Relation between anemia prevalence in **<u>men</u>** and urinary cadmium (U-Cd) levels and renal dysfunction markers

Men		Anemia	Non-anemia	Total	Chi-square
		N (%)	N (%)	N (%)	p-value
U-Cd (µg/g Cr)	=<2	1 (7.69)	12 (92.31)	13 (100.00)	0.228*
	2-5	17 (24.64)	52 (75.36)	69 (100.00)	
	5-10	23 (19.66)	94 (80.34)	117 (100.00)	
	>10	19 (30.65)	43 (69.35)	62 (100.00)	
β_2-MG (µg/g Cr)	<400	21 (14.09)	128 (85.91)	149 (100.00)	<0.001
	400-1000	9 (27.27)	24 (72.73)	33 (100.00)	
	>1000	30 (37.97)	49 (62.03)	79 (100.00)	
NAG (Unit/g Cr)	<4	6 (6.82)	82 (93.18)	88 (100.00)	<0.001
	4-8	30 (26.09)	85 (73.91)	115 (100.00)	
	>8	24 (41.38)	34 (58.62)	58 (100.00)	
Cystatin C (mg/l)	<0.9	5 (9.80)	46 (90.20)	51 (100.00)	<0.001
	0.9-1.4	27 (17.42)	128 (82.58)	155 (100.00)	
	>1.4	28 (50.91)	27 (49.09)	55 (100.00)	
Total		60 (22.99)	201 (77.01)	261 (100.00)	

U-Cd = 2 µg/g Cr or lower was considered as a safe reference level; β_2-MG >1,000 µg/g Cr indicated irreversible proximal tubular dysfunction; NAG>8 unit/g Cr indicated proximal tubular cell damage; Cystatin C >1.4 mg/l indicated glomerular dysfunction
*analyzed with Chi-square and Fisher exact tests

Table 20 Relation between anemia prevalence in **women** and urinary cadmium (U-Cd) levels, and renal dysfunction markers

Women		Anemia N (%)	Non-anemia N (%)	Total N (%)	Chi-square p-value
U-Cd (µg/g Cr)	=<2	5 (20.83)	19 (79.17)	24 (100.00)	0.034
	2-5	21 (22.58)	72 (77.42)	93 (100.00)	
	5-10	37 (18.32)	165 (81.68)	202 (100.00)	
	>10	39 (32.50)	81 (67.50)	120 (100.00)	
β_2-MG (µg/g Cr)	<400	61 (18.60)	267 (81.40)	328 (100.00)	<0.001
	400-1000	11 (26.83)	30 (73.17)	41 (100.00)	
	>1000	30 (42.86)	40 (57.14)	70 (100.00)	
NAG (Unit/g Cr)	<4	13 (10.92)	106 (89.08)	119 (100.00)	<0.001
	4-8	49 (24.50)	151 (75.50)	200 (100.00)	
	>8	40 (33.33)	80 (66.67)	120 (100.00)	
Cystatin C (mg/l)	<0.9	26 (17.22)	125 (82.78)	151 (100.00)	<0.001
	0.9-1.4	51 (22.37)	177 (77.63)	228 (100.00)	
	>1.4	25 (41.67)	35 (58.33)	60 (100.00)	
Total		102 (23.23)	337 (76.77)	439 (100.00)	

U-Cd = 2 µg/g Cr or lower was considered as a safe reference level; β_2-MG >1,000 µg/g Cr indicated irreversible proximal tubular dysfunction; NAG>8 unit/g Cr indicated proximal tubular cell damage; Cystatin C >1.4 mg/l indicated glomerular dysfunction
*analyzed with Chi-square and Fisher exact tests

Table 21 Relation between anemia prevalence and β_2-MG, NAG levels after adjusted by means age and urinary cadmium (U-Cd) among the study subjects

	Men			Women			Total		
	Expected Odds	95.0% C.I.		Expected Odds	95.0% C.I.		Expected Odds	95.0% C.I.	
		Lower	Upper		Lower	Upper		Lower	Upper
R^{2a}	0.23			0.07			0.11		
Age	1.07***	1.04	1.10	1.01	0.99	1.03	1.03***	1.02	1.05
log U-Cd	2.68	0.80	8.91	1.48	0.64	3.41	1.80	0.92	3.54
β_2-MG <400	1.00			1.00			1.00		
β_2-MG 400-1,000	1.58	0.59	4.19	1.44	0.68	3.09	1.47	0.82	2.63
β_2-MG >1,000	1.61	0.77	3.35	2.77***	1.56	4.92	2.25***	1.45	3.49
R^{2a}	0.24			0.05			0.12		
Age	1.07***	1.04	1.09	1.02	1.00	1.03	1.03***	1.01	1.04
log U-Cd	2.10	0.63	7.04	1.43	0.63	3.25	1.46	0.74	2.88
NAG <2.5	1.00			1.00			1.00		
NAG 2.5-8	2.79	0.35	22.31	1.98	0.67	5.86	2.63***	1.51	4.56
NAG >8	4.81	0.55	41.98	3.28*	1.05	10.26	3.71***	2.03	6.79

The relation was analyzed by binary logistic regression model between anemia prevalence and age, log U-Cd, β_2-MG, and NAG. Anemia prevalence was set as dependent whereas age, log U-Cd, β_2-MG, and NAG were explanators. β_2-MG <400, and NAG <2.5 values were used as reference and their expected odds ratio was set as 1. [a]Nagelkerke R Square values, *$p<0.05$, **$p<0.01$, ***$p<0.001$

Table 22 Relation between anemia prevalence and cystatin C levels after adjusted by means age and urinary cadmium (U-Cd) among the study subjects

	Men			Women			Total		
	Expected Odds	95.0% C.I.		Expected Odds	95.0% C.I.		Expected Odds	95.0% C.I.	
		Lower	Upper		Lower	Upper		Lower	Upper
R^{2a}	0.25			0.05			0.11		
Age	1.06***	1.03	1.10	1.01	0.99	1.03	1.03**	1.01	1.05
log U-Cd	2.85	0.86	9.44	1.86	0.81	4.25	2.16*	1.10	4.23
Cystatin C <0.9	1.00			1.00			1.00		
Cystatin C 0.9-1.4	0.69	0.21	2.21	1.20	0.67	2.16	0.95	0.57	1.59
Cystatin C >1.4	1.59	0.40	6.29	2.86*	1.27	6.44	2.40**	1.23	4.67

$$\text{Odds ratio} = \frac{\text{Ratio of disease/non-disease in exposure group}}{\text{Ratio of disease/non-disease in non-exposure group}}$$

The relation was analyzed by binary logistic regression model between anemia prevalence and age, log U-Cd, and Cystatin C. Anemia prevalence was set as dependent whereas age, log U-Cd, and cystatin C were explanators. Cystatin C >0.90 mg/l was used as reference value and its expected odds ratio was set as 1.

[a]Nagelkerke R Square values, *p<0.05, **p<0.01, ***p<0.001

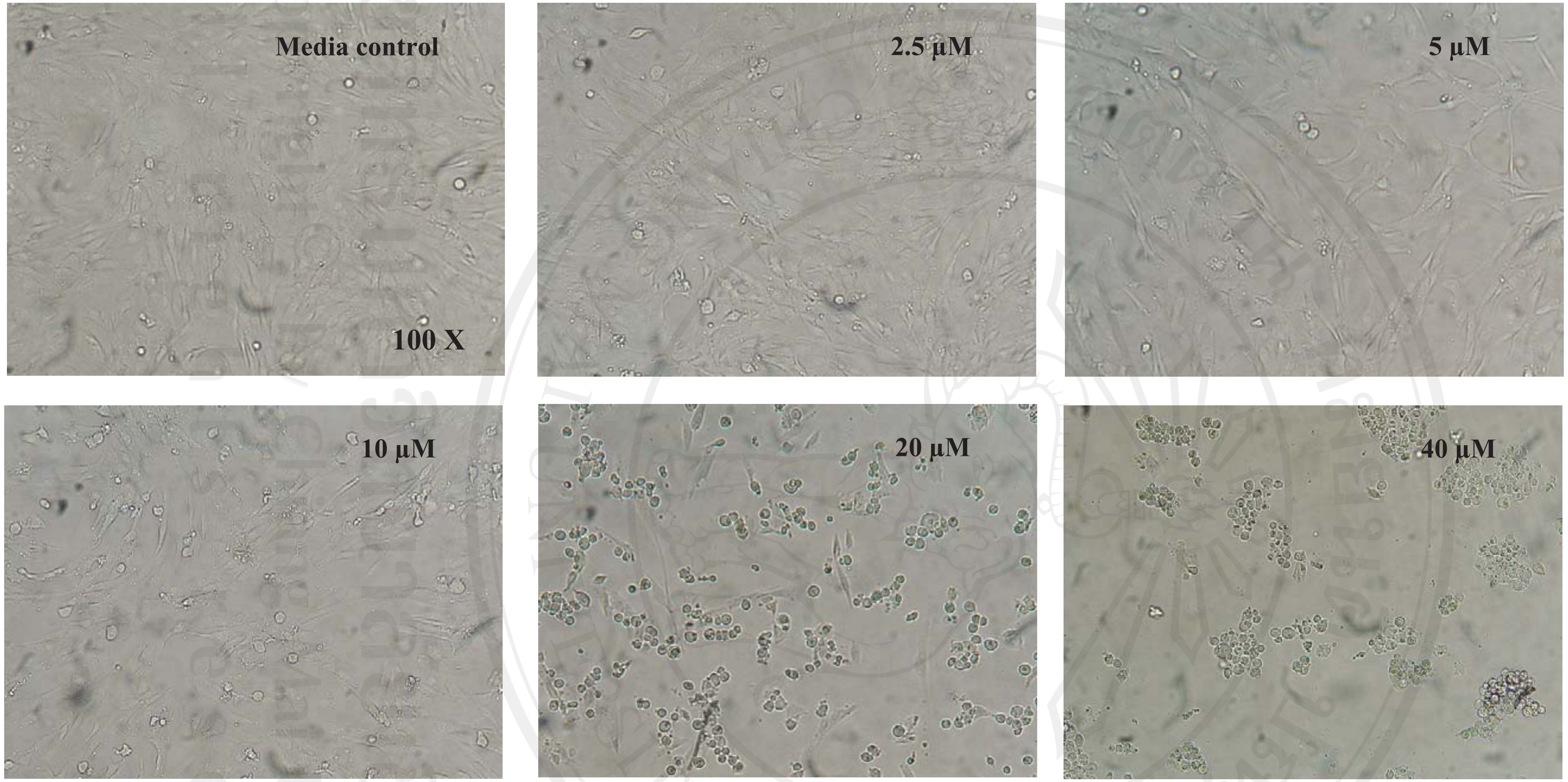

Figure 11 Morphology of human fetal osteoblast like cells (hFOB 1.19 cells) (100X) treated with $CdCl_2$ concentrations 2.5, 5, 10. 20 and 40 µM compared to the untreated cells (midia control)

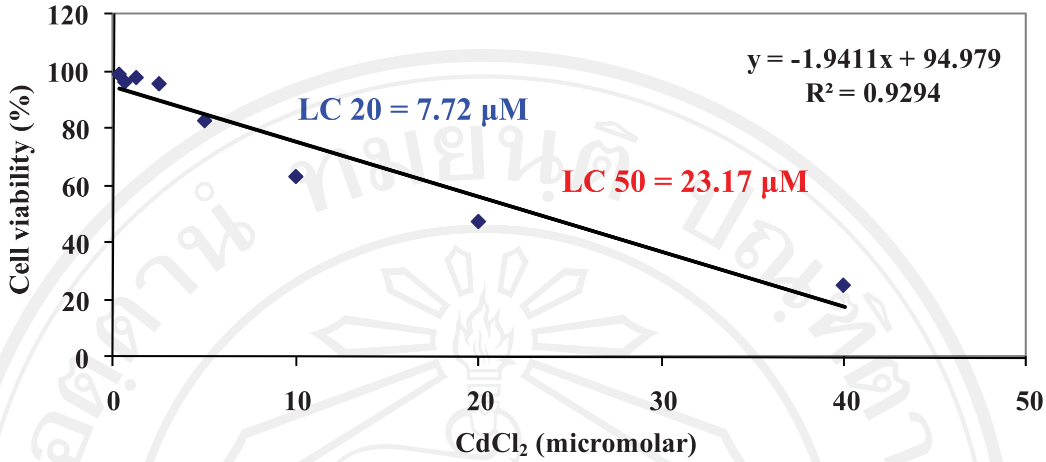

Figure 12 Cytotoxicity of cadmium chloride ($CdCl_2$) in human fetal osteoblasts like cells (hFOB 1.19) after 24 h treatment using MTT assay, the data expressed as %cell survival compared to non-treated cells and calculated for lethal concentrations, LC20 and LC50

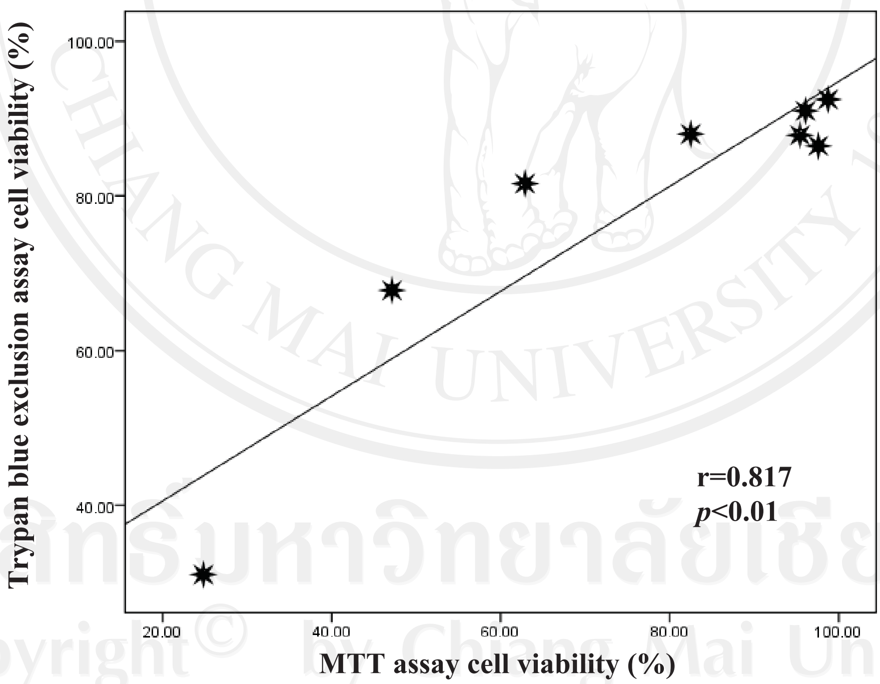

Figure 13 Correlation of %hFOB cell viability between MTT and trypan blue exclusion assays after treatment with $CdCl_2$ solution, r = Spearman rho's correlation coefficient.

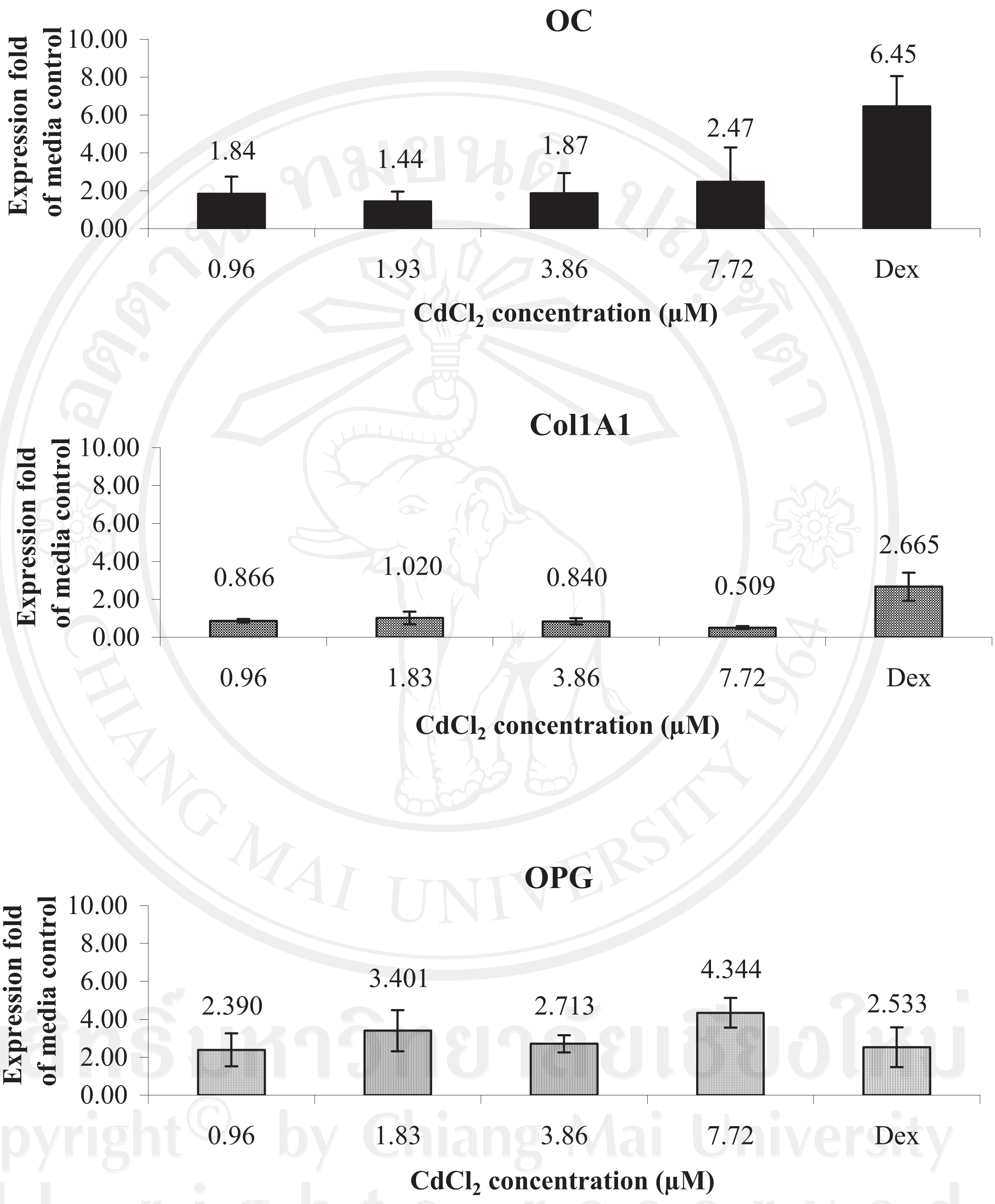

Figure 14 Expression of osteocalcin, typeI collagen and OPG genes of the human fetal osteoblast like cells (hFOB cell) after 24 hr treatment with $CdCl_2$ concentrations of 0.96, 1.83, 3.86 and 7.72 µM and 10^{-6} M of dexamethasone (Dex) compared to the results from media control[148].