Open Journal of Nephrology, 2013, 3, 205-210
Published Online December 2013 (http://www.scirp.org/journal/ojneph)
http://dx.doi.org/10.4236/ojneph.2013.34035

Twenty-Two-Year Observation on Urinary Cadmium and ß$_2$-Microglobulin in Inhabitants after Cessation of Cadmium-Exposure in Japan*

Reiko Sato[1#], Teruhiko Kido[1], Hideaki Nakagawa[2], Muneko Nishijo[2], Ryumon Honda[2], Etsuko Kobayashi[3], Yasushi Suwazono[3]
[1]Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan
[2]Kanazawa Medical University, Uchinada, Japan
[3]Graduate School of Medical Science, Chiba University, Chiba, Japan
Email: #totosuki2007@yahoo.co.jp

Received October 22, 2013; revised November 20, 2013; accepted December 15, 2013

ABSTRACT

Objective: This is an epidemiological survey that was conducted for 22 years to investigate the physical effects on inhabitants who had been exposed to cadmium (Cd) from processing of remnants discharged at the time of copper refinement. It was possible to obtain findings on physical effects 27 years after Cd exposure. **Materials and Methods:** Of the inhabitants who were 50 years old or younger in 1981 and who were living in the most contaminated area in the Kakehashi River basin, 68 (32 males and 36 females) who underwent all of the 5 screenings during the 22-year period were extracted as subjects. Early morning urine was collected for urinalysis, and Cd and β_2-MG concentrations were determined. **Results:** 27 years after cessation of Cd exposure, it was shown that urinary Cd concentrations were significantly decreased and urinary β_2-MG concentrations were significantly increased. **Conclusion:** Once exposed to Cd, it takes about 30 years for the Cd that remains in the body to decrease by half. Once renal tubular dysfunction occurs after Cd exposure, irreversible aggravation is inevitable.

Keywords: Twenty-Two-Year Observation; Urinary-Cadmium; Urinary-β_2-Microglobulin; Itai-Itai Disease; Biological-Half-Life

1. Introduction

A long-term follow-up survey was conducted to investigate the effects of Cd exposure on the health of inhabitants who had been living in a contaminated region continually in Japan with regard to the following: 1) Cd that remained in the body as determined based on changes in urinary Cd concentration [1], and 2) renal tubular disorder [2], a typical consequence of Cd exposure, based on changes in urinary β_2-microglobulin (β_2-MG) [3,4] concentration.

Once exposed to Cd, about 30 years [5,6] are required to reduce by half the Cd that remains in the body regardless of the amount of exposure. Even after a long period of time, β_2-MG, an index of renal tubular dysfunction caused by exposure, continues to irreversibly increase [7-10] instead of decreasing. It has therefore been suggested that with larger exposure volumes, the risk of development of distressing Itai-itai disease [11-13] may increase infinitely.

The Kakehashi River basin located in Komatsu City, Ishikawa Prefecture, Japan, was contaminated with Cd waste water from the former Ogoya Cupper Mine [14] from 1882 to 1971. The inhabitants of the region were orally exposed to Cd [15] for a long time because they cultivated and consumed rice as a staple food with contaminated water used for irrigation in rice paddies. However, the Ishikawa Prefecture government conducted an health impairment investigation only afterward, in the 1970s.

The physical effects of Cd exposure include renal tubular dysfunction, osteomalacia, osteoporosis, hepatic disorder, hypertension [16], and others. The most severe consequence is Itai-itai disease. The name of this disease

*The authors declare no competing financial interests.
#Corresponding author.

is Japanese, and literally means "ouch-ouch" because even small movements result in fractures due to advanced osteomalacia, making the patient cry (According to autopsy reports, 42 fractures were observed in some cases.).

The Ministry of Health and Welfare of Japan recognized Itai-itai disease as the first mining-related illness in April 1968 [17] and subsequently enacted a law to improve the polluted soil. Based on this law, in 1981 Ishikawa Prefecture improved the soil of the Kakehashi River basin district that was contaminated with Cd. Since the Ogoya Mine had already been closed by this time and its operation suspended, it was declared that Cd exposure had discontinued in this region [18]. At the same time in 1981, the prefectural government investigated health effects [19] only among inhabitants of the Kakehashi River district who were 50 years old or older at that time and also believed to be severely exposed to Cd. In the survey, findings of β_2-MG of 1000 μg/g·Cr [20,21] or higher were judged abnormal. The incidence of abnormal values was 14.3% in males and 18.7% in females who lived in the polluted area, and 6% and 5%, respectively, in those who lived in non-polluted areas, clearly demonstrating a significant difference ($p < 0.01$) [22] between the polluted and non polluted areas. However, the inhabitants who were 50 years of age or younger and assumed to be moderately exposed to Cd were not investigated by Ishikawa Prefecture even though they lived in the same polluted Kakehashi River area. During 22 years from 1986, 5 years after the declaration on the discontinuation of exposure, up to 2008, Kido *et al.* who were concerned about the pollution, conducted a survey 5 times (1986, 1991, 1999, 2003 and 2008) [23,24] to investigate health effects using only excluded inhabitants who were 50 years of age or younger at the time of the former survey. Our study summarizes these investigations.

The objectives of the studies were 1) to investigate the changes in urinary Cd and β_2-MG concentrations in males and females over the 22-year period to determine the excretion of these substances and 2) to determine the relationship between Cd exposure and renal tubular dysfunction on the basis of the correlation of urinary Cd with β_2-MG in males and females over the 22 years. The following methods were used.

2. Materials and Methods

Of all the inhabitants who were 50 years old or younger in 1981 and who were living in the commune, which was one of the most contaminated areas in the Kakehashi River basin, 68 (32 males and 36 females) who under-went all of the 5 screenings during the 22-year period were included as subjects. Inhabitants who neither participated at least once due to their illness or moving were excluded. Early morning urine was collected for urinalysis, and Cd and β_2-MG concentrations were determined. The measured Cd and β_2-MG concentrations were corrected with Cr. As methods of determination, RIA (Radioimmunoassay), flameless atomic absorption spectrophotometry [25] and the Jaffe method were used to determine β_2-MG concentration, Cd concentration, and Cr concentration, respectively. A questionnaire was also used to investigate gender, age, and duration of residence.

β_2-MG is a plasma protein of low molecular weight (11.800 Daltons) and is produced from immunologically competent cells, the liver, and other tissues. Almost 100% of β_2-MG is filtered through the glomeruli of the kidneys and 99.9% of that is reabsorbed in the renal tubules [24-26]. Accordingly, β_2-MG was used as an index of renal tubular dysfunction. Currently, new indexes of renal tubular dysfunction are being described. However, the newest and the most sensitive index as of 1986 was urinary β_2-MG [24,25]. In a long-term follow-up epidemiology survey, it was necessary to employ the same investigation method in the same subjects for 22 years. Therefore, urinary β_2-MG was continuously used as an index of renal tubular dysfunction for the 22-year period.

The results were statically analyzed using analysis of covariance (ANCOVA), Bonferroni and Pearson correlation coefficient. The software used for analysis was SPSS12 OJ for Windows.

The inhabitants participated in the survey of their own free will. The survey in 2008 was granted approval by the Ethics Committee of Kanazawa University (2008, approval No. 166).

3. Results and Discussion

The mean ages of subjects in 2008 were 65 years and 68 years in the case of males and females, respectively. The mean years of residence were 53 years and 46 years, respectively (**Table 1**).

The scales of **Figures 1**-**3** show concentrations converted to logarithmic values.

Figure 1 shows a graph of average urinary Cd concentrations of 32 males and 36 females during the 22-year period. On t-testing of the difference in average Cd concentrations of male and female urine specimens in 1986 and 2008, the levels of significance were females $p = 0.001$ and males $p = 0.001$, indicating a significant decrease in 2008. The average actual measurements in 1986 were males 6.0 μg/g·Cr and females 8.20 μg/g·Cr, while the 2008 average actual measurements were males 2.30 μg/g·Cr and females 5.70 μg/g·Cr. This graph reflects the biological half-life of Cd, which is about 30 years, though the results also suggest long-term residue of Cd in the body even 27 years after exposure. In addition, the

Table1. Information on tested subjects (Males = 32, Females = 36).

	1986		1991		1999		2003		2008	
	M	F	M	F	M	F	M	F	M	F
Average age	43	46	48	50	56	59	60	62	65	68
Average period of residence	31	24	36	29	44	37	48	41	53	46

M: Males; F: Females.

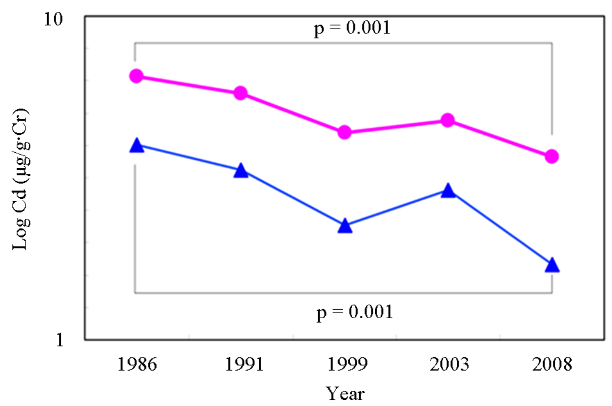

Figure 1. Changes in urinary cadmium concentration (μg/g·Cr) over 22 years in 32 males (Blue) and 36 females (Red).

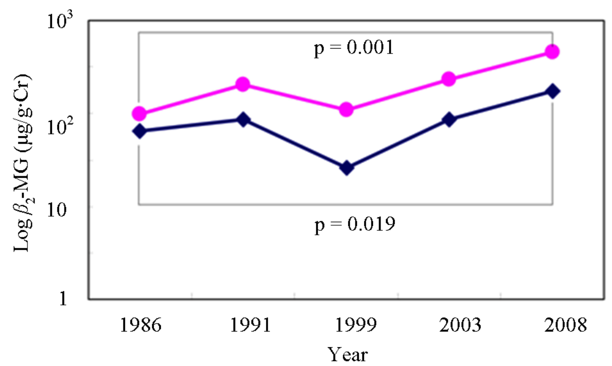

Figure 2. Changes in urinary β_2-MG concentration (μg/g·Cr) over 22 years in males (Blue) and females (Red).

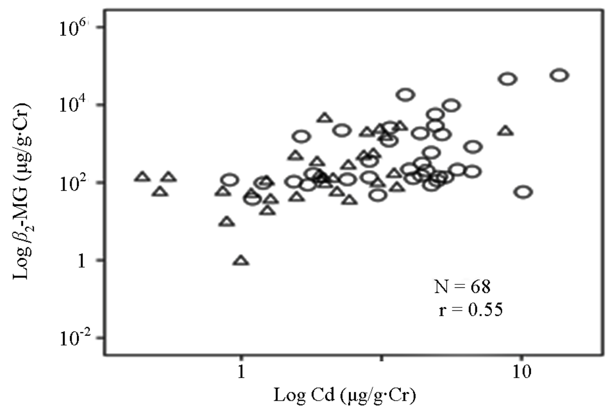

Figure 3. Scatter-plot of correlation between urinary Cd and ß2-MG concentrations for 36 females (○) and 32 males (△) in 2008 (r = 0.55: Pearson correlation coefficient, p = 0.000).

mean concentration in females was clearly higher than that in males, since Cd absorption rate depends on the metallic ion. In conditions of iron deficiency in particular, the absorption rate increases by 20 to 30% compared with normal. The higher incidence of iron deficiency anaemia observed in females [26,27] due to menstruation, giving birth, etc. is cited as a reason why Cd concentration was higher in females than males.

Figure 2 shows a graph of average urinary β_2-MG concentrations of 32 males and 36 females during 22 years. The average actual measurements in 1986 were males 181.0 μg/g·Cr and females 199.0 μg/g·Cr, while the 2008 values were males 224.0 μg/g·Cr and females 268.0 g/g·Cr. On t-testing of the difference in average Cd concentrations in male and female urine specimens in 1986 and 2008, levels of significance were females p = 0.001 and males p = 0.019. The level significantly increased in both males and females.

These results indicate that, once renal tubular dysfunction occurs after Cd exposure, no cure can be expected and irreversible aggravation is inevitable over time. Fortunately, however, the subject group consisted of those moderately exposed rather than severely exposed to Cd. Since the mean β_2-MG concentration at the time of initial screening in 1986 was 1000 μg/g·Cr or less [18], these individuals had not suffered renal tubular dysfunction, with the exception of several individuals, even though a significant increase in the β_2-MG concentration was noted after 22 years (see **Figure 3**).

In addition, the downward trend in average concentration shown in **Figures 1** and **2** in 1999 is interpreted as the result of physiologically fluctuation, since all values were within normal [24].

To adjust the effects of age or period of residence on the trend in average concentrations of urinary Cd and β_2-MG, analysis of covariance (ANCOVA) analysis was performed and results were shown from **Tables 2-5**. **Table 2** shows the change of urinary Cd concentration during 22 years adjusted by age in males and females. Preliminary parallel tests showed a positive result for females and a negative result for males. Hence, the

Table 2. The change of urinary Cd concentration during 22 years adjusted for age in females.

Urinary Cd (μg/g·Cr)						
Year	Females (N = 36)					
	GM	SE	p			
1986	0.968	0.48		p = 0.002		
1991	0.857	0.046		p = 0.001	p = 0.027	
1999	0.615	0.045	p = 0.001			
2003	0.601	0.045		p = 0.000		p = 0.049
2008	0.419	0.048				

GM: Geometric Mean; SE: Standard Error; p: p-value; A Parallel test for males was negative and positive for females. Hence, a further Bonferroni multiple comparison in ANCOVA analysis was performed for females.

Table 3. The change of urinary Cd concentration during 22 years adjusted for period of residence in males and females.

Urinary Cd (μg/g·Cr)									
	Males (N = 32)				Females (N = 36)				
Year	GM	SE	p		GM	SE	p		
1986	0.670	0.058	p = 0.001		0.925	0.050		p = 0.039	
1991	0.563	0.056		p = 0.000	0.824	0.048			
1999	0.343	0.055	p = 0.038		0.626	0.047	p = 0.000	p = 0.038	
2003	0.430	0.056	p = 0.000	p = 0.012	0.622	0.048		p = 0.000	
2008	0.170	0.057			0.464	0.049			

GM: Geometric Mean; SE: Standard Error; p: p-value; Parallel tests for males and females showed a positive result. Hence, a further Bonferroni multiple comparison in ANCOVA analysis was performed.

Table 4. The change of urinary ß$_2$-MG concentration during 22 years adjusted for age in males and females.

Urinary ß$_2$-MG (μg/g·Cr)									
	Males (N = 32)					Females (N = 36)			
Year	GM	SE	p			GM	SE	p	
1986	1.979	0.131	p = 0.16			2.306	0.121		
1991	2.025	0.126		p = 0.005		2.510	0.116	p = 0.014	
1999	1.394	0.124				1.983	0.113		
2003	1.863	0.125			p = 0.001	2.212	0.114		
2008	2.090	0.130				2.377	0.120		

GM: Geometric Mean; SE: Standard Error; p: p-value; Parallel tests for males and females showed a positive result. Hence, a further Bonferroni multiple comparison in ANCOVA analysis was performed.

resulting Bonferroni multiple comparison in ANCOVA analysis for females showed a significant decrease during 22 years (1986 - 2008), without involvement of age. In **Table 3** the change of urinary Cd concentration during 22 years was adjusted by period of residence in males and females. Preliminary parallel tests showed a positive result for both males and females. In the resulting in ANCOVA and Bonferroni multiple comparison analysis, there was a significant decrease in males and females during 22 years (1986 - 2008), without the involvement period of residence. As shown in **Table 4** the change of urinary ß$_2$-MG concentration during 22 years was adjusted by age and preliminary parallel tests showed a positive result in males and females. Hence, the resulting Bonferroni multiple comparison in ANCOVA analysis showed a significant decrease between 1986 to 1999 and

Table 5. The change of urinary β_2-MG concentration during 22 years adjusted for Period of residence in males and females.

Urinary β_2-MG (μg/g·Cr)						
	Males (N = 32)			Females (N = 36)		
Year	GM	SE	p	GM	SE	p
1986	1.935	0.130	p = 0.038 (1986–1991)	2.267	0.118	
1991	2.008	0.126	p = 0.009 (1991–1999)	2.468	0.114	p = 0.038 (1991–1999)
1999	1.902	0.125		1.999	0.112	
2003	1.877	0.126	p = 0.001 (1999–2003)	2,230	0.113	
2008	2.129	0.129		2.424	0.117	

GM: Geometric Mean; SE: Standard Erro; p: p-value; A Parallel test for males and females showed a positive result. Hence, a further Bonferroni multiple comparison in ANCOVA analysis was performed.

1991 to 1999, but there was significant increase during 1999 to 2008 in males, with no significant increase in females. However, there was a tendency towards increase during 22 years (1986 - 2008) for both males and females without the involvement of age. **Table 5** shows that urinary β_2-MG concentration during 22 years was adjusted by period of residence. A significant increase was observed between 1999 and 2008 for males, but for females, there was no significant increase without the involvement of period of residence. However, during 22 years (1986 - 2008) there was a tendency to increase in males and females. Therefore, Significant increase were observed for urinary β_2-MG concentration (μg/g·Cr) adjusted by age and period of residence, for males and females. An increasing tendency was observed during 22 years for females. It was revealed that once exposed to Cd, β_2-MG occurs in the body and irreversible

Figure 3 shows a scatter-plot of the correlation between urinary Cd and β_2-MG concentrations for 36 females (○) and 32 males (△) in 2008 using the Pearson correlation method. This figure indicates a significant correlation of urinary Cd with β_2-MG in 2008 with a Pearson correlation coefficient of $r = 0.55$ and a significance level of $p = 0.0001$. The relationship of Cd exposure to renal tubular dysfunction indicates that this dysfunction persisted even 27 years after Cd exposure and as long as Cd remained in the body.

4. Conclusion

Our findings clearly indicate the following. Once exposed to Cd, it takes about 30 years for the Cd that remains in the body to decrease by half. Once renal tubular dysfunction occurs after Cd exposure, irreversible aggravation is inevitable. If the Cd exposure volume is larger, the renal tubular dysfunction will be more severe. Therefore, measures to prevent mining pollution are required for mining development in the 21st century so that the tragedy caused by Cd pollution that occurred as a secondary effect of cupper mining development is never repeated. In addition, the first measures to prevent mining pollution is a process of gravel remaining emissions during the refining process. The gravel that contain contaminants is discharged, and appropriate processing of it is the first step in preventing pollution. However, we may be able to find rare metals by carefully sifting this gravel, which has been irresponsibly discharged.

5. Acknowledgements

The authors thank the inhabitants of the Kakehashi area, who had to endure physical examinations over a period of 22 years. Special thanks go to Mr. M. Iga, a historian of Kakehashi town, who is currently 96 years of age. The authors also thank Mr. M. Hino for environmental data on the Kakehashi river stream (Komatsu Environmental Partnership) and Mr. H. Hayashidera.

Finally, we sincerely wish the inhabitants of the Kakehashi area good health and long lives.

6. Author Contributions

Dr. Sato conducted the project. Dr. Nishijo, Dr. Kobayashi, and Dr. Honda analyzed the urinary samples. Dr. Kido and Dr. Nakagawa participated in several health examinations. Dr. Suwazono calculated analytical data.

REFERENCES

[1] T. Kido, "The Relation of Individual Cadmium Concentration in Urine with Total Cadmium Intake in Kakehashi River Basin, Japan," *Toxicology Letters*, Vol. 152, No. 1, 2004, pp. 57-61. http://dx.doi.org/10.1016/j.toxlet.2004.04.001

[2] T. KjellstrÖm, "Renal Effects," In: L. Fridberg, C. G. Elinder, T. KjellstrÖm and G. F Nordberg, Eds., *Cadmium and Health*: *A Toxicological and Epidemiological*

Appraisal, Ⅱ. *Effects and Response*, CRC Press, Boca Raton, 1986, pp. 21-110

[3] P. A. Peterson, P. E. Evrin and I. Berggård, "Differentiation of Glomerular, Tubular and Normal Proteinuria: Determinations of Urinary Excretion of β_2-MG, Albumin and Total Protein," *Journal of Clinical Investigation*, Vol. 48, 1969, pp. 1189-1198.

[4] M. Piscator, "Markers of Tubular Dysfunction," *Toxicology Letters*, Vol. 46, 1989, pp. 197-204. http://dx.doi.org/10.1016/0378-4274(89)90128-8

[5] K. Nogawa, "Itai-Itai Disease and Follow-Up Studies," In: J. O. Nriagu Ed., Cadmium in the Environment Part 2 Health Effects," A Wiley-Interscience Publication, New York, 1981, I-37.

[6] K. Aoshima and M. Kasuya, "Itai-Itai Disease a Natural History of Osteomalacia Caused by Urinary Tubular Dysfunction during Exposure to General Environmental Cadmium," *The Journal of Therapy*, Vol. 75, 1993, pp. 1031-1035.

[7] World Health Organization, "Environmental Health Criteria 135 Cadmium—Environmental Aspects," World Health Organization, Geneva, 1992, pp. 174-188.

[8] G. F. Nordberg, *et al.*, "Cadmium and Health. A Toxicological and Epidemiological Appraisal. Vol. I," CRC Press, Boca Raton, 1985, pp. 103-178.

[9] G. F. Nordberg, *et al.*, "Cadmium," In: L. Friberg, C.-G. Elinder, T. KjellstrÖm and G. F. Nordberg, Eds., *Hand Book on the Toxicology of Metals*, 3rd Edition, Elsevier, Amsterdam, 2007.

[10] C.-G. Elinder, "Cadmium and Health. A Toxicological and Epidemiological Appraisal. Chapter 3," CRC Press, Boca Raton, 1985.

[11] K. Iwata, H. Saito, M. Moriyama and A. Nakano, "Renal Tubular Function after Reduction of Environmental Cadmium-Exposure. A Ten-Year Follow up," *Arch Environ Health*, Vol. 43, No. 3, 1993, pp. 157-163. http://dx.doi.org/10.1080/00039896.1993.9940814

[12] G. Nordberg, "Renal Dysfunction Induced by Cadmium-Is It Reversible? Advance in Prevention of Environmental Cadmium Pollution and Countermeasures," *Eiko Laboratory Kanazawa JAPAN*, 1999, pp. 99-102.

[13] Y. Cai, K. Aoshima, T. Katoh, H. Teranishi and M. Kasuya, "Renal Tubular Dysfunction in Male Inhabitants of Cadmium-Polluted Areas in Toyama, Japan-An Eleven-Year Follow-Up Study," *Journal of Epidemiology*, Vol. 11, No. 4, 2001, pp. 180-189. http://dx.doi.org/10.2188/jea.11.180

[14] T. Wada, "Metals and Humans," Asakura Publishers, 1986, pp. 246-258.

[15] K. Nogawa and A. Ishizaki, "Statistical Observations of the Does-Response Relationship of Cadmium Based on Epidemiological Studies in the Kakehashi River Basin," *Environmental Research*, Vol. 15, 1978, pp. 185-198. http://dx.doi.org/10.1016/0013-9351(78)90095-6

[16] M. Kasuya, "Liver Injury by Cadmium," *Kan Tan Sui*, Vol. 10, 1985, pp. 923-928.

[17] J. Matsunami, "Review of Itai-Itai Disease," Katsura Syobou, Japan. 2006, pp. 2-38.

[18] Ishikawa Prefecture Laws and Regulation, "Measurement of Soil in Farmlands of Polluted Areas," Ishikawa Prefecture, 1975. www.pre.ishikawa.jp/reiki/reiki_honbun/enkaku/i1010488001,html

[19] Ishikawa Prefecture South Kaga Public Health Center, "Research and Induction about Local Health and Environmental during 9 Heisei Year (1997)," Report on Ishikawa Prefecture South Kaga Public Health, 1984, pp. 82-88.

[20] K. Nogawa, Y. Yamada, R. Honda, M. Ishizaki, I. Tsuritani, S. Kawano and T. Kato, "The Relationship between Itai-Itai Disease among Inhabitants of the Jinzu River basin in rice," *Toxicology Letters*, Vol. 17, No. 3, 1983, pp. 263-266. http://dx.doi.org/10.1016/0378-4274(83)90236-9

[21] Y. Kubota, *et al.*, "A Study on Normal Range of Urinary Protein Fraction Concentrations, Albumin, β2-Microgroblin and α1-Microgrobulin among Commounity Inhabitants," *Hokuriku Journal of Public Health*, Vol. 12, 1985, pp. 27-32.

[22] T. Kido, "Studies on Health Effects of Cadmium Exposure in the General Environment," *Japanese Journal of Hygiene*, Vol. 49, No. 6, 1995, pp. 960-972. http://dx.doi.org/10.1265/jjh.49.960

[23] T. Kido, "Progress of Renal Dysfunction in Inhabitants Environmentally Exposed to Cadmium," *Archives of Environmental Health*, Vol. 43, No. 3, 1988, pp. 214-215. http://dx.doi.org/10.1080/00039896.1988.9934935

[24] R. Sato, T. Kido, R. Honda, M. Nishijo, H. Nakagawa, E. Kobayashi and Y. Suwazono, "Seventeen-Year Observation on Urinary Cadmium and β2-Microglobulin in Inhabitants after Cessation of Cadmium-Exposure in Japan," *Bull Environ Contam Toxicol*, Vol. 84, 2010, pp. 363-367. http://dx.doi.org/10.1007/s00128-010-9947-6

[25] R. Honda, *et al.*, "Flameless Atomic Absorption Spectrophotoemetry of Cadmium and Lead in Blood," *Kanazawa Medical University Journal*, Vol. 14, No. 3, 1989, pp. 337-347.

[26] P. A. Peterson, P. E. Evrin and I. Berggård, "Differential of Glomerular, Tubular and Normal Proteinuria: Determinations of Urinary Excretion of β2-MG, Albumin and Total Protein," *Journal of Clinical Investigation*, 1969, pp. 1189-1198.

[27] G. F. Nordberg, L. Friberg, C. Elinder and T. Kjellstrom, "Cadmium and Health. A Toxicological and Epidemiological Appraisal. Vol. 1," CRC Press, Boco Raton, 1985, pp. 103-178.