ORIGINAL ARTICLE

Low cadmium concentration in whole blood from residents of Northern Sardinia (Italy) with special reference to smoking habits

R. MADEDDU, E. MURESU[*], A. MONTELLA[**], G. CHESSA[***], G.B. CHERCHI[****], P. PIRAS[***], P. VARGIU[****], P. TOLU[**], A. PIRINO[**], J.C. PRADOS[*****], P. CASTIGLIA[*]

Department of Biomedical Sciences and Istituto Nazionale Biostrutture e Biosistemi, University of Sassari; [*] Institute of Hygiene, Faculty of Medicine University of Sassari; [**] Department of Biomedical Sciences, University of Sassari; [***] Istituto Zooprofilattico della Sardegna, Sassari; [****] Analysis Laboratory, S.S. Annunziata Hospital ASL N. 1, Sassari, Italy; [*****] Department of Anatomy, Faculty of Medicine, University of Granada, Spain

Key words

Cadmium • Blood • Smoking habit

Summary

Introduction. *The present study was initiated to investigate the cadmium concentrations in whole blood of Northern Sardinian, non-occupationally exposed adult subjects. Sardinia is a large Italian island which differs genetically and environmentally from other mainland Italian areas.*

Methods. *Two hundred and forty-three adults (157 females and 86 males) were selected in the study area from subjects who were undergoing blood collection for laboratory analysis during the period January 2005-May 2005. Whole blood was analysed by graphite furnace atomic absorption spectrometer equipped with a Zeeman-effect background corrector (Perkin Elmer ZL5100) and an auto sampler. The adopted analytical procedure uses the Stabilized Platform Temperature Furnace (STPF) technique.*

Results. *The mean value of Blood Cadmium Concentration (BCdC), expressed as Geometric Mean, was 0.32 μg/l (CI 95%: 0.31-0.34 μg/l) significantly ranging from 0.27 μg/l (CI 95%: 0.26-0.29 μg/l) in non-smokers to 0.34 μg/l (CI 95%: 0.30-0.39 μg/l) in ex-smokers up to 0.47 μg/l (CI 95%: 0.42-0.53 μg/l) in smokers ($p < 0.0001$).*

Discussion. *The results show that BCdC levels in Northern Sardinian non-occupationally exposed adults are lower than levels found in many other regions, including those within Italy. Nevertheless, similar values have been detected in other European countries and cities.*

Conclusions. *In relation to other reports in which data were analysed by strata for smoking habit and age, we found similar BCdC values among non smokers. However, Sardinian smokers seem to show lower levels of blood cadmium.*

Introduction

Cadmium (Cd) is a toxic heavy metal which is present in the environment. The extremely long biological half life (30-35 years) makes it a cumulative toxin [1], so long-term past exposure could still result in direct toxic effects [2].

Even if natural sources of cadmium do exist (volcanic activity, forest fires and windblown transport of soil particles), the main source for the general population consists of environmental pollution by human activity [3-6].

Major occupational exposure occurs in non-ferrous metal smelters, in the production and processing cadmium, in the production of Ni-Cd batteries and, increasingly, in the recycling of electronic waste [7, 8].

Non-occupational exposure is mainly from cigarette smoke, which contains relatively high concentrations of the metal; for non-smokers who are not exposed in non-occupational activities such as particular hobbies (welding, painting, gardening, etc.), diet is the main route [3, 9-11].

Cadmium has a long biological half-time, and it is mainly accumulated in liver and kidneys. IARC, the International Agency for Research on Cancer, classified cadmium as a carcinogen (group I). Several studies supported a relationship between cadmium exposure and human cancer (lung, kidney, prostate), but an increasing number of studies are tending to indicate a likely association with human cancer of the liver, bladder, breast, brain and of the haematopoietic system [12-18].

Several studies have shown the relationship between chronic exposure to cadmium and blood pressure in animals and humans [19-21]. The need to determine background levels of trace elements in biological samples to discern possible trends of environmental pollutants on human health has been recognised [22].

So far, various studies have been conducted on the concentration of Cadmium in whole blood, which is considered a good biomarker and reflects recent exposure to the metal under conditions of moderate exposure; while over longer periods of time, urine is a preferred biomarker [8].

Regarding Europe, several studies have been made on the general population and on particular clusters of residents, especially in Scandinavia, Germany, Slovenia, Spain and the Czech Republic [23-30].

There is also a lot of data from America and Asia, especially from China and Japan where increased exposure has been reported [31-35].

In Italy, several studies have been carried out in the North of the country, while few data are available from central and Southern regions. To our knowledge, no studies have been performed in the insular region of Sardinia [30, 36-38]. Sardinia can be distinguished from other Italian regions by characteristics determined by its insularity: industrialization is only present in localized zones and population differs genetically from that of mainland. In fact, Sardinia has long been an object of study for particular genetic characteristics which are linked or correlated to peculiar diseases, for example talassemia, multiple sclerosis and diabetes [39-43].
A study on the concentration of Cadmium in the whole blood of residents of Northern Sardinia, who are not occupationally exposed, associated to some variables such as smoke, age, sex will report the first pattern on cadmium exposure of the general population in this area and could represent a source of useful data both for preventive purposes and for future research in medical fields.

Materials and methods

Subjects and area

The present study was performed in the health district of Sassari, an area in Northern Sardinia with approximately 340,000 inhabitants. It is well known that the Sardinian population has a genetic background very different to the rest of Italy and all known European and African populations [44]. The Sardinian population is genetically quite homogeneous and because of its insularity, external influence in term of migratory flux is moderate, making it well adapted for epidemiological purposes.
Two hundred and forty-three adults (157 females and 86 males) were selected in the study area from subjects who were undergoing blood collection for laboratory analysis during the period January 2007-May 2007. The mean age was 50.0 years (standard deviation 18.6). There were 143 non-smokers, 39 ex-smokers (i.e., smokers which have stopped smoking for 6 months) and 61 current smokers. The exclusion criteria were: age under 18 years and/or a high exposure to cadmium related to occupation or hobby. Subjects were enrolled as participants in the study only after they had yielded informed consent to participate.
An anonymous questionnaire for each participant was compiled concerning demographic characteristics, residence in the last 15 years, occupation, hobbies, diet and smoking habit.

Sampling and analysis

For cadmium determination, a venous blood sample of 5 ml at fasting was collected in EDTA-containing tubes. Blood samples, with identification numbers corresponding to the same questionnaires, were immediately frozen at –20°C in Nunc crio-vials until analysis.
Cadmium measurements were performed by graphite furnace atomic absorption spectrometer equipped with a Zeeman-effect background corrector (Perkin Elmer ZL5100) and a auto sampler. The adopted analytical procedure uses the Stabilized Platform Temperature Furnace (STPF) technique.
Samples to be analysed were withdrawn from the refrigerator and kept overnight at room temperature. One hundred microliters aliquots of each blood sample were added to 900 μl of an aqueous solution containing 0.01% Triton X-100, and carefully mixed in clean metal-free plastic tubes. The solutions were then transferred into the vials of the auto sampler. Chemical modifier solution was prepared by mixing 2000 mg.l^{-1} of $NH_4H_2PO_4$ and 207 mg.l^{-1} di $Mg(NO_3)_26H_2O$. Five microliters of this solution were added to every aliquot of measurement solution in the graphite tube.
The instrumental method required that two aliquots of sample (20 μl) and two aliquots of chemical modifier solution (5 μl) were added to the furnace. After each sample introduction, a dried step was performed. This step allows an increase in the amount of sample analysed and improves the detection limit. Determinations of cadmium were performed by interpolation of the sample peak area with a calibration curve. Calibration was carried out by using aqueous solution of cadmium at the following concentrations: 0.02, 0.04 and 0.08µg/l. The working solutions, containing 0.01% Triton X-100, were prepared daily.
The regression equation was calculated using unweighted least squares regression analysis. The curve parameters were: slope, $b = 0.177 \pm 0.005$; intercept, $a = -0.0002 \pm 0.0002$; determination coefficient, $r^2 = 0.998$. The calibration curve passes through the origin of the axis.
The limit of detection (LoD) was 0.05 µg/l, evaluated with the Upper Limit Approach (ULA2) related to the calibration curve.
Reliability estimation method was performed by analysing two incurred blood samples at a Cadmium concentration of 0.30 µg/l and 0.67 µg/l (expressed as mean value). The replicates, six for each sample, provide the following coefficients of variation: 17% for the sample at 0.30 µg/l and 12% for the sample at 0.67 µg/l. The accuracy of the method was assessed against the certified reference material BCR 194, purchased from Community Bureau of Reference, Brussels, Belgium, with certified Cadmium concentration of 0.20 µg/l ± 0.05 µg/l. The mean value obtained, with six replicates, was 0.25 µg/l (SD, 0.01 µg/l). All materials employed in collection, storing and analysis of blood samples were of the highest quality and certificated. Water was deionized and then further purified with a Milli-Q system (Millipore). Collection tubes were warmed up with a nitric acid solution wash.

Statistical methods

Statistical analysis was performed using Stata 9® [45]. Do to the asymmetric distribution of data, a log transformation was applied. Confidence Intervals (CI 95%) for geometric mean (GM) were calculated according to the appropriate *t* distribution degrees of freedom. Differ-

ences among groups were tested via variance analysis. A linear regression model was also applied to study dependence of blood cadmium concentration (BCdC) on explicative variables. The median and percentiles of BCdC were also calculated. A p-value of < 0.05 (two tails) was chosen to indicate statistical significance; if statistical test did not reach significance, a "NS" response (Not Significant) was set. Participants were grouped into categories of age: < 40 i.e., 18 to 39 years, 40-59 i.e., 40 to 59 years and > 59 i.e., over 59 years old. Smoking history was grouped into three categories: non-smokers, ex-smokers and smokers. Smokers were further classified as light smokers (1-10 cigarettes/day), moderate smokers (11-20 cigarettes/day) and heavy smokers (> 20 cigarettes/day).

Results

The distribution of data was considerably asymmetric (Fisher's γ_1: 1.51) and log transformation was able to make it symmetric (Fisher's γ_1: 0.15). The BCdC ranged from 0.10 μg/l to 1.2 μg/l.

Table I reports the distribution of BCdC by smoking habit. One hundred forty-three subjects (58.8%) were non-smokers, 39 (16.1%) were ex-smokers and 61 were smokers (25.1%). The overall geometric mean (GM) of BCdC was 0.32 μg/l (CI 95%: 0.31-0.34 μg/l) significantly ranging (ANOVA: $p < 0.0001$) from 0.27 μg/l (CI 95%: 0.26-0.29 μg/l) in non-smokers to 0.34 μg/l (CI 95%: 0.30-0.39 μg/l) in ex-smokers up to 0.47 μg/l (CI 95%: 0.42-0.53 μg/l) in smokers. Because of the efficiency of log transformation the median values of BCdC in the whole set (0.31 μg/l) and in specific subgroups by smoking habit (0.27, 0.33, 0.50 μg/l, respectively) are similar to GM ones. The increasing trend detected in BCdC-GMs and in BCdC-median values through the three categories of non-smokers, ex-smokers and smokers was also observed in percentile values.

No significant differences in BCdC-GMs between genders (males: GM = 0.33 μg/l; CI95%: 0,29-0.36; females: GM = 0.32 μg/l; CI 95%: 0.30-0.34) were detected. Slightly higher values were detected in non-smoker and ex-smoker females, but not in smokers where males seem to have higher values (Tab. II). Moreover, the increasing trend through the three categories of smoking habits was observed in both sexes ($p < 0.0001$).

On the whole, no significant differences in BCdC-GMs were observed among categories of age (Tab. III). However, when data were stratified by smoking habit, significant differences by age arose in non-smokers ($p < 0.05$). In that category, BCdC resulted depending on age (linear regression of log-values: intercept = –1,60, coeff. B = 0,0061; $p < 0,001$) (Tab. IV). Nevertheless significant differences ($p < 0.0001$) were observed in mean age among categories of smoking habit (Tab. V), with ex-smokers the oldest group (59.5 ± 16.2) and smokers the youngest (43.4 ± 13.8). Table VI reports the distribution of BCdC-GMs in smokers by number of cigarettes/day. As expected, an increase in BCdC-GMs was observed among categories of smokers with values ranging from 0.44 μg/l in light smokers (CI 95%: 0.38-0.49 μg/l) to 0.48 μg/l in moderate smokers (CI 95%:

Tab. I. BCdC (μg/l) in relation to smoking habit.

Smoking habit			95% GM-CI						
	N	GM*	lower	upper	Median	p5	p25	p75	p95
Non-smokers	143	0.27	0.26	0.29	0.27	0.13	0.21	0.36	0.53
Ex-smokers	39	0.34	0.30	0.39	0.33	0.20	0.26	0.42	0.61
Smokers	61	0.47	0.42	0.53	0.50	0.21	0.38	0.61	0.86
Total	243	0.32	0.31	0.34	0.31	0.15	0.24	0.45	0.74

N: sample size; GM: geometric mean; 95% GM-CI: confidence interval for GM; p5, p25, p75, p95: percentiles; * significant parameter: analysis of variance $p < 0.0001$.

Tab. II. BCdC (μg/l) in relation to gender and smoking habit.

Smoking habit	Males				Females				p
			95% GM-CI				95% GM-CI		
	N	GM*	lower	upper	N	GM*	lower	upper	
Non-smokers	34	0.25	0.22	0.28	109	0.28	0.26	0.31	NS
Ex-smokers	22	0.32	0.27	0.39	17	0.36	0.30	0.43	NS
Smokers	30	0.47	0.41	0.55	31	0.44	0.35	0.55	NS
Total	86	0.33	0.29	0.36	157	0.32	0.30	0.34	NS

N: sample size; GM: geometric mean; 95% GM-CI: confidence interval for GM; NS: not significant ($p > 0.05$); * significant parameter: analysis of variance $p < 0.0001$.

Tab. III. BCdC (μg/l) in relation to age and smoking habit.

Age	Non smokers				Ex smokers				Smokers				Total			
			95% CI				95% CI				95% CI				95% CI	
	N	GM	L	U	N	GM	L	U	N	GM	L	U	N	GM	L	U
<40	50	0.23	0.09	0.58	5	0.35	0.21	0.58	27	0.43	0.15	1.38	82	0.30	0.10	0.92
40-59	39	0.28	0.13	0.68	16	0.31	0.11	0.88	26	0.40	0.26	0.87	81	0.32	0.14	0.84
>59	54	0.29	0.13	0.67	18	0.33	0.17	0.77	8	0.51	0.19	1.34	80	0.31	0.15	0.72
Total	143				39				61				243			
p		<0.05				NS				NS				NS		

N: sample size; GM: geometric mean; 95% GM-CI: confidence interval for GM; L: lower; U: upper limits; NS: not significant ($p > 0.05$).

Tab. IV. Regression model for log-trasformed BCdC in non smokers.

					95% Confidence Intervals	
Variable	Coeff.	Std. Err.	t-value	p-value	Lower	Upper
Age	0.0061	0.0017	3.60	0.000	0.0027	0.0094
Constant	–1.6011	0.0910	–17.60	0.000	–1.7810	–1.4214
Model R-squared = 0.08.						

Tab. V. Age in relation to smoking habit.

			Age*	
Smoking habit	N	GM*	Mean	St. dev.
Non-smokers	143	0.27	50.0	20.0
Ex-smokers	39	0.34	59.5	16.2
Smokers	61	0.47	43.4	13.8
Total	243	0.32	49.9	18.7

*significant parameter: analysis of variance p < 0.0001.

Tab. VI. Distribution of BCdC-GMs (μg/l) in smokers, classified by number of smoked cigarettes/day

Category of smokers, n. cigarettes/day			95% Confidence Intervals	
	N	GM	lower	upper
Light, 1-10	24	0.44	0.38	0.49
Moderate, 11-20	31	0.48	0.40	0.57
Heavy, > 20	6	0.64	0.50	0.80
Total	61			

0.40-0.57 μg/l) up to 0.64 μg/l in heavy smokers (CI 95%: 0.50-0.80 μg/l), although these differences were not significant because of the small size of the last category (p = 0.16).

Discussion

The results of the present study, concerning BCdC in general adult population not occupationally exposed residing in Northern Sardinia (overall GMs 0.32 μg/l, ranging from 0.10 μg/l to 1.20 μg/l), show that the BCdC is lower than levels reported in other Italian populations [30, 36, 37] which ranged between 0.5 μg/l to 0.7 μg/l. Higher values were also detected in European regions [25, 26, 28, 46] and in Asia, particularly in Japan [31, 35]. Nevertheless similar values have been detected in other European countries and cities [24, 47] and in Asia, where in Singapore it ranges from 0.21 μg/l to 0.49μg/l [33]. The BCdC results depend on smoking habit, with increasing levels both through overall ordinal categories (from non-smokers to smokers) and through specific ones (from light smokers to heavy smokers). These results in non-smokers (0.27 μg/l), in ex-smokers (0.34 μg/l) and in smokers (0.47 μg/l), with a level about two times higher in the latter category compared to the first one, emphasize the role of cigarette smoking in enhancing BCdC. This finding, in accordance with other reports [3, 25, 36, 48-50] shows that alongside diet, cigarette smoking is the most important behavioural determinant for BCdC in non-occupationally exposed population. Moreover, this figure is confirmed in our study by the increasing BCdCs detected in smokers regarding consumption of cigarettes per day: 0.44 μg/l in light smokers, 0.48 μg/l in moderate smokers and 0.64 μg/l in heavy smokers. Several studies report similar data [3, 36] even if conflicting results are reported: in the Singaporeon study, where BCdC was analyzed in three ethnic groups, in Chinese group non-smokers had higher concentration than light and moderate smokers: 0.15 μg/l, 0.10 μg/l and 0.10 μg/l, respectively [33]. Furthermore, in our study, there was a non-homogeneous distribution of data set by age and smoking habit, as smokers were significantly younger than other categories, and an early initiation of smoking was found in Sardinian people [51, 52]. The possible additive effect of cadmium accumulation by age might have interfered with smoking habit; in fact, we found that BCdCs depend on age in non-smokers (linear regression: p < 0.001) but not in the categories of ex-smokers and smokers, where the effect of cigarette smoking hides that of the age. Thus, the unbalanced distribution of the sample by age and categories of smokers triggers off an overall slight increase of BCdCs with age.

Gender doesn't seem to be a contributing factor for BCdCs, 0.33 μg/l in males and 0.32 μg/l in females, as has also been reported in several other studies [26, 28], although we found a moderate but not significantly higher BCdC-GMs in non-smoking and former smoker women, as already described in Sweden [3]. On the other hand, in Morocco, it was reported that cadmium concentration in men was 30% higher than in women overall [49] and similar results were found in Northern Italy [30]. On the whole, even if it has been reported that cadmium exposure may influence mortality in gender [53], we believe that gender is not a true determinant for BCdCs and the conflicting results obtained in different reports may depend on confounding.

In conclusion, overall BCd levels in Northern Sardinian non-occupationally exposed people were lower than

many other areas. Nevertheless, this aggregate result may be affected by a non-homogeneous distribution of the sample by strata of behavioural variables referring to other studies. In fact, in relation to other reports, when data were analysed by strata for smoking habit and age we found similar values among non smokers, but Northern Sardinian smokers seem to show lower levels of blood cadmium. In part, these results may reflect short-term exposure since we used blood instead of urine concentration as a biomarker, whereas urine is the preferred biomarker for long-term exposure.

These low values may be explained in part by environmental factors considering that the study was conducted in an area at low risk from industrial cadmium fall-out. Nevertheless, in view of the peculiar characteristics of the Sardinian population, other factors that may influence BCd levels, like dietary and genetic factors [33], could be further investigated.

References

[1] Yilmaz O. *Cadmium and lead levels in human liver and kidney samples obtained from Bursa Province*. Int J Environ Health Res 2002;12:181-5.

[2] Goering PL, Waalkes MP, Klaasen CD. *Toxicology of cadmium*. In: Goyer RA, editor. *Handbook of experimental pharmacology: toxicology of metals. Biochemical effects*. New York: Springer-Verlag 1994:115, 189-214.

[3] Jarup 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;25:645-56.

[4] Thuvander B, Oskarrson A. *Adverse health effects due to soil and water acidification*. The national environmental protection agency, Stockolm, report 4881, 1997.

[5] Sartor FA, Rondia DJ, Claeys FD, Staessen JA, Lauwerys RR, Bernard AM, et al. *Impact of environmental cadmium pollution on cadmium exposure and body burden*. Arch Environ Health 1992;46:347-53.

[6] Nomiyama K. *Recent progress and perspectives in cadmium health effect studies*. Sci Total Environ 1980;14:199-232.

[7] Bacio A, Nadal M, Garcia F, Domingo JL. *Monitoring metals in the population living in the vicinity of a hazardous waste incinerator: concentrations in autopsy tissues*. Biol Trace Element Res 2005;106:41-50.

[8] Lauwerys RR, Hoet P. *Industrial chemical exposure: Guidelines for biological monitoring* 2nd Ed. Boca Raton: Lewis Publishers 1993:32-8.

[9] Satarug S, Baker JR, Urbenjapol S, Hasswell-Elkins M, Reilly PEB, Williams DJ, et al. *A global perspective on cadmium pollution and toxicity in non-occupationally exposed population*. Toxicol Lett 2003;137:65-83.

[10] Watenabe T, Zhang ZW, Moon CS, Shimbo S, Nakatsuka H, Matsuda-Inoguchi N, et al. *Cadmium exposure of women in general populations in Japan during 1991-1997 compared with 1977-1981*. Int Arch Occup Environ Health 2000;73:26-34.

[11] Bernard A, Lauwerys R. *Cadmium in human population*. Experientia 1984;40:143-52.

[12] Waalkes MP. *Cadmium carcinogenesis*. Mut Res 2003;533:107-20.

[13] Nakamura K, Yasunaga Y, Ko D, Xu LL, Moul JW, Peehl DM, et al. *Cadmium induced-neoplastic transformation of human prostate epithelial cells*. Int J Oncol 2002;20:543-7.

[14] Achanzar WE, Diwan BA, Quader ST, Webber MM, Waalkes MP. *Cadmium-induced malignant transformation of human prostate epithelial cells*. Cancer Res 2001;61:455-8.

[15] Al-Saleh I, Shinwari N. *Levels of cadmium, lead and mercury in human brain tumors*. Biol Trace Element Res 2001;79:197-203.

[16] Pesch B, Haerting J, Ranft U, Klimpel A, Oelschlagel B, Schill W. *MURC Study group. Occupational risk factors for renal cell carcinoma agent specific results from a case control study in Germany*. Int J Epidemiol 2000;29:1014-24.

[17] Nordberg GF. *Cadmium carcinogenesis and its relationship to other health effects in humans*. Scand J Work Environ Health 1993;19(Suppl.1):104-7.

[18] International Agency for Research on Cancer (IARC) Monographs. *Beryllium Cadmium Mercury in the glass industry*. Vol. 58. Lyon: IARC 1993:119-238.

[19] Loyke HF. *Effects of elements in human blood pressure control*. Biol Trace Element Res 2002;85 :193-209.

[20] Wang S, Paek D, Kim R, Cha B. *Variation of systolic blood pressure in rats exposed to cadmium and nickel*. Environ Res 2002;88:116-9.

[21] Vivoli G, Bergomi M, Borella P, Fantuzzi G, Caselgrandi E. *Cadmium in blood, urine and hair related to human hypertension*. J Trace Elem Electrolytes Health Dis 1989;3:139-45.

[22] Ogunfowokan AO, Famuyiwa SO, Adenuga AA, Fatoki OS. *Determination of cadmium and lead in urine of some Nigerian subjects*. Int J Environ Health Res 2002;12:283-7.

[23] Wilhelm M, Ewers U, Schulz C. *Revised and new reference values for some traces elements in blood and urine for human biomonitoring in environmental medicine*. Int J Hyg Environ Health 2004;207:69-73.

[24] Erzen I, Zaletel Krageli L. *Exposure assessment of male recruits in Slovenia to cadmium and lead due to biological monitoring*. J Expo Anal Environ Epidemiol 2004;14:3855-90.

[25] Becker K, Kraus S, Krause C, Lepom P, Schulz C, Seiwert M, et al. *German environmental survey (GerES III): environmental pollutants in blood of the german population*. Int J Hyg Environ Health 2002;205:297-308.

[26] Benes C, Spevackova V, Smid J, Cejchanova M, Cerna M, Subrt P, et al. *The concentrations levels of Cd, Pb, Hg, Cu and Se in blood of the population in the Czech republic*. Cent Eur J Publ Health 2000;8:117-9.

[27] Nordberg M, Winblad B, Basun H. *Cadmium concentration in blood in an elderly urban population*. Biometals 2004;13:311-7.

[28] Moreno MA, Marin C, Vinagre F, Ostapczuch P. *Trace element levels in whole blood samples from residents of the city Badajoz, Spain*. Sci Total Environ 1999;229:209-15.

[29] Herber RFM, Christensen JM, Sabbioni E. *Critical evaluation and review of cadmium concentrations in blood for use in occupational health according to TRACY protocol*. Int Occup Environ Health 1997;69:372-8.

[30] Alessio L, Apostoli P, Braga M, Duca PG, Herber RFM, Nordberg G, et al. *Estimation of pooled reference values for cadmium in blood using meta-analysis and TRACY criteria*. Sci Total Environ 1994;152:169-77.

[31] Zhang ZW, Moon CS, Watanabe T, Shimbo S, He FS, Wu YQ, et al. *Background exposure of urban population to lead and cadmium: comparison between China and Japan*. Int Arch Environ Health 1997;69:273-81.

[32] Watanabe T, Nakatsuaka H, Shimbo S, Iwami O, Imai Y, Moon CS, et al. *Reduced cadmium and lead burden in Japan in past 10 years*. Int Arch Occup Environ Health 1996;68:305-14.

[33] Chia SE, Chan OY, Sam CT, Heng BH. *Blood cadmium levels in non-occupationally exposed adult subjects in Singapore*. Sci Total Environ 1994;145:119-23.

[34] Benedetti JL, Dewailly E, Turcotte F, Lefebre M. *Unusually high blood cadmium associated with cigarette smoking among three subgroups of the general population*. Sci Total Environ 1994;152:161-7.

[35] Watanabe T, Qu JB, Jin C, Liu YT, Yin SN, Nakatsuaka H, et al. *Blood cadmium levels in the population of 3 Chinese cities*. Toxicol Lett 1989;47:145-53.

[36] Dell'Omo M, Muzi G, Piccinini R, Gambelunghe A, Morucci P, Fiordi T, et al. *Blood cadmium concentrations in the general population of Umbria, Central Italy*. Sci Total Environ 1999;226:57-64.

[37] Roggi C, Sabbioni E, Minoia C. *Trace element reference values in tissues from inhabitants of the European Union. IX Harmonization of statistical treatment: blood cadmium in Italian subjects*. Sci Total Environ 1995;166:235-43.

[38] Minoia D, Sabbioni E, Apostoli P. *Trace elements reference values in tissues from inhabitants of the European community. A study of 46 elements in urine, blood and serum of Italian subjects*. Sci Total Environ 1990;95:89-105.

[39] Pugliatti M, Solinas G, Sotgiu S, Castiglia P, Rosati G. *Multiple sclerosis distribution in northern Sardinia: spatial cluster analysis of prevalence*. Neurology 2002;58:277-82.

[40] Sotgiu, S, Pugliatti M, Solinas G, Castiglia P, Sanna A, Rosati G. *Immunogenetic heterogeneity of multiple sclerosis in Sardinia*. Neurol Sci 2001;22:167-70.

[41] Rosatelli MC, Dozi A, Faa V, Meloni A, Sardu R, Saba L, et al. *Molecular characterization of beta-thalassemia in the Sardinian population*. Am J Hum Genet 1992;50:422-6.

[42] Contu L, Carcassi C, Trucco M. *Diabetes susceptibility in Sardinia*. Lancet 1991;338:8758- 65.

[43] Masala B, Manca L, Gallisai D, Stangoni A, Lanclod KD, Kutlar F, et al. *Biochemical and molecular aspects of beta-thalassemia types in Northern Sardinia*. Haemoglobin 1988;12:661-71.

[44] Cavalli-Sforza L, Menozzi P, Piazza A. *The history and geography of human genes*. Princeton: Princeton University Press 1994:272-6.

[45] STATA Corp. Stata Statistical Software Release 9. College Station, TX: StataCorp LP. 2005.

[46] Puklova V, Batariova A, Cerna M, Kotlik B, Kratzer K, Melichercik J, et al. *Cadmium exposure pathway in the Czech urban population*. Cent Eur J Public Health 2005;13:11-9.

[47] Friberg L, Vahter A. *Assessment of exposure to lead cadmium through biological monitoring: result of a UNEP/WHO global study*. Environ Res 1983;30:95-128.

[48] Gokmen IG, El-Agha O. *Smoking habits and cadmium intake in turkey*. Biol Trace Element Res 2002;88:31-44.

[49] Khassouni CE, Soulaymani R, Maura Y, Allain P. *Blood cadmium concentration in the population of the Rabat area, Morocco*. Clin Chim Acta 2000;302:155-60.

[50] Ewers U, Brockhaus A, Dolgner R., Freier I, Turfeld M, Engelke R, et al. *Blood lead and blood cadmium concentration in 55-66-year-old women from different areas of Nordrhein-Westfalen-chronoligal trends during 1982-1988*. Zentralbl Lyg Umweltmed 1990;189:405-18.

[51] Maida A, Solinas G, Masia MD, Diana M, Dettori M, Pirastu R, et al. *Epidemiological survey on smoking habit among young students in Sardinia. A cross sectional study*. Ann Ig 2005;17:209-17.

[52] Masia MD, Solinas G, Piana A, Dettori M, Sotgiu G, Castiglia P. *Smoking habit and behaviour among health professionals*. Ann Ig 2006;18:261-9.

[53] Uetani M, Kobayashi E, Suwazono Y, Kido T, Nogawa K. *Cadmium exposure aggravates mortality in woman than in men*. Int J Environ Health Res 2006;16:273-9.

■ Received on June 27, 2008. Accepted on February 4, 2009.

■ Acknowledgements: This work was supported by a research grant from the Banco di Sardegna Foundation. The authors are grateful to Dr G. Padula, Dr S. Masia, Dr A. Gadeddu, Dr M. Cossu, Dr G. Simula and Drssa G. Nieddu for their support and co-operation.

■ Correspondence: Dr Roberto Madeddu, Department of Biomedical Sciences and Istituto Nazionale Biostrutture e Biosistemi, University of Sassari, viale S. Pietro 43/B, 07100 Sassari - Tel. +39 079 228569 - Fax +39 079 228523 - E-mail: rmadeddu@uniss.it