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Cadmium Concentrations in Blood in a Group of Male Recruits in Slovenia Related to Smoking Habits

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The intake of certain harmful substances is considered to be a substantial health risk, even when the concentrations of such substances are relatively low, which is mostly due to a longer exposure period. This particularly applies to substances, which tend to accumulate in the body, like cadmium (Satarug and Moore 2002; EHC 1992). Human exposure to cadmium has received a lot of attention during the past several decades; many studies have shown that, beside occupationally exposure, dietary intake and, in particular, tobacco smoking are important for higher intake of to this nephrotoxic element (Satarug et al. 2004; Cerná et al. 2001). Several authors have reported that cadmium concentrations in blood (Cd-B) are higher in smokers than in non-smokers (Olsson et al. 2002; Weyermann and Brenner 1997). In moderately exposed persons, the usual Cd-B is typically less than 1μg/L, however it can be up to 4μg/L (Watanabe et al. 1996). The level also depends on whether such persons are smokers or non-smokers or subject to passive smoking. Among smokers, the mean Cd-B was found to be two times higher compared to that in non-smokers; this however depends on the amount and brand of cigarettes smoked (Shaham et al. 1996; Staessen et al. 1990).

In Slovenia, a Central European country (2 million inhabitants, 21 000 square km), there were no data available for an in-depth assessment of Cd-B in the population until 2001. However data from a study performed on a small group of blood donors (N=64), occupationally non-exposed to increased cadmium intake (Eržen and Zaletel Kragelj 2004) was available. The cadmium level was low in the blood of non-smokers (median = 0.3 μg/L), whereas in the blood of smokers, the median Cd-B was as high as 0.9 μg/L.

MATERIALS AND METHODS

The study is a part of a wider study, aimed at evaluating the cadmium and lead burdening in young males, occupationally not exposed to both pollutants in Slovenia (Eržen and Zaletel Kragelj 2003). It was performed on the total population of military recruits called up for military service in Slovenia between August and November 2001. They were invited to participate in the study at the time of obligatory vaccination against tick-borne meningoencephalitis prior to the starting of the military service (the call-up of recruits in Slovenia took place randomly),

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which was performed at the domicile Regional Public Health Institute.

Taking into account the expected number of recruits and data on the prevalence of smoking among the young population, we determined that study's enrollment period should last at least 4 months. The response to our invitation was excellent; out of 792, as many as 742 subjects, which are 93.7%, decided to participate.

The participants were asked to fill in a questionnaire about their age, profession, leisure-time activities and behaviors related to health like nutrition, alcohol consumption and smoking habits. The smoking status of participants was assessed based on five questions:

1. "Do you smoke?" (possible answers: I don't smoke and I never have; I don't smoke now but I smoked in the past; yes, I smoke),
2. "When did you stop smoking- write date or at least year in which you stopped smoking",
3. "If you smoke, how many cigarettes you smoke per day-write the number",
4. "Do you smoke cigar or pipe" (possible answers: yes, I do or no, I don't), and
5. "How many cigars or pipes do you smoke per day? -write the number".

On the basis of these questions the participants were classified in three groups: 1) never smokers and past smokers (being only three, and all stopped smoking more than five years prior to the study), 2) light to moderate smokers (smoking of maximum 20 cigarettes/day), and 3) heavy smokers (smoking of 21 or more cigarettes/day).

In order to be able to assess the cadmium intake due to cigarette smoking as accurately as possible, our later analyses excluded everyone who drank more than 20g of alcohol a day, had special eating habits (vegetarians, vegans) or could be occupationally exposed to increased cadmium intake. Working in mines, glass or metal (especially color-metal) industry or as a painter was considered possible occupational exposure to cadmium.

Finally, there were 708 participants included in the analysis of differences in Cd-B between non-smokers and smokers. It was assumed that the dietary cadmium intake was normally distributed between all participants and that Cd-B level in non-smokers represents the level of dietary cadmium intake.

Blood samples up to 2 ml were taken by means of a 5 ml syringe with added EDTA from the cubital vein. Prior to sample taking, the skin was thoroughly wiped with a disinfectant (70% alcohol or Cetavlon). Each blood sample was given its identification number, equal to the number on the questionnaire completed by the participant, prior to blood sample taking. In the cases where analysis did not take place within 48 hrs, the blood samples were frozen and stored at – 20 degree C. Prior to being analyzed, blood samples were diluted with the solution of 0.25% ascorbic acid and 1% Triton X-100, applied ratio 1:5. The samples were analyzed directly by means of electro thermal atomic absorption spectroscopy (ETAAS) (SpectrAA-20, Varian, Mulgrave, Australia). For the blowing of graphite tube atomizers, argon gas was used. During the ashing stage, the air was introduced into

the graphite furnace instead of argon, in order to burn the organic matrix. The Cd absorbance was at 228.8 nm. The background correction was performed by employing the deuterium lamp. The limit of quantification (LOQ) for Cd-B, which was defined as ten times the standard deviation of replicate measurements of independent control blanks, was 0.5 μg/L. The 5-point calibration took place prior to sample measurement and afterwards at the interval of every ten samples. In such a manner, it was possible to ensure that all samples and standards were prepared in the same way and with the same chemicals. For the calibration, the method of standard addition was applied, with palladium used as a modifier (0.1 g/L). The blank sample was prepared in the same manner.

Each blood sample was prepared and analyzed in duplicates. The final value represents the mean value of both measurements. Based on ten measurements of different paired samples, the standard deviation was calculated. The reliability of measurements was considered satisfactory when the difference between parallel determinations did not exceed two standard deviations. In case of greater difference, however, the measurements were repeated.

For monitoring the correctness of blood analysis method, the BCR CRM 194 certified reference material was used (Institute of Reference Materials and Measurements, Geel, Belgium). The analysis results for reference materials were satisfactory (observed values 94-103% of certified value, variation coefficient 4.7%).

The Cd-B values can be regarded as quantitative or semi quantitative, depending on how the values below the LOQ are treated. In our study, the following procedure was used for the imputation of data below the LOQ: to the values lower than the LOQ, the value of one third of the LOQ was assigned.

In statistical description, both the parametric and nonparametric method was applied. The parametric method (mean) was applied in order to obtain results comparable to those of other studies using the same kind of statistical analysis, while the non-parametric method (median, interquartile range, 10^{th} and 90^{th} percentiles) was used because the values observed were not quantitative in all cases. We considered the non-parametric method as the main one. Overall differences among non-smokers and smokers of different levels were assessed using the Kruskal-Wallis test, a non-parametric analogue to the analysis of variance, while multiple paired comparisons were assessed using Mann-Whitney test, a non-parametric analogue to Student t-test (15). In assessing the overall differences, the level of statistical significance was set to $p<0.05$, while in paired comparisons the Bonferroni correction method was used, taking into account two paired comparisons ($p=0.05/2=0.025$). SPSS statistical package for Windows (Version 11.0, SPSS Inc, Chicago, IL, USA) was used for analysis.

The participants were informed on the purpose and course of our study and they all entered the study voluntarily. The study entirely complies with Helsinki Declaration. In addition, the Ethical Committee of the Republic of Slovenia approved the basic study in 1999.

RESULTS AND DISCUSSION

There were 384 non-smokers (54.2%), 217 light to moderate smokers (30.6%), and 107 heavy smokers (15.1%) among participants of the study. The age varied between 18 and 27 with the mean being 20.5±1.9 years.

The distribution of typical values CD-B values of all three observed groups are presented in Figure 1. In non-smokers the Cd-B values were relatively low. The median value was 0.50 μg/L and inter-quartile range: 0.15-0.50 μg/L (mean level was 0.76 μg/L). In light to moderate smokers the Cd-B values were significantly higher than in non-smokers. The median value was 1.00 μg/L, inter-quartile range: 1.00-2.00 μg/L (mean level was 1.58 μg/L). In heavy smokers the Cd-B values were the highest. . The median level was 1.50 μg/L, inter-quartile range: 1.00-2.00 μg/L (the mean level was 1.76μg/L).

In contrast to the group of non smokers, in the group of heavy smokers the proportion of those who had Cd-B lower than LOQ was 1.9 % of all participants placed in this group. Also the proportion of those who had Cd-B up to 1 μg/L was low, being 17.6% of participants placed in this group (Table 1). It is interesting, that the proportion of those who had Cd-B up to 1 μg/L comparing to the group of non smokers was quite low in both different groups of smokers: 22.4% and 19.4% in group of light to moderate smokers, and heavy smokers respectively. In the group of non smokers this proportion was as big as 73.9%. The overall differences in Cd-B between non-smokers, light to moderate smokers and heavy smokers assessed using the Kruskal-Wallis test, were statistically highly significant ($p<0.001$). Multiple comparisons showed that the difference between non-smokers and light to moderate smokers assessed using Mann-Whitney test was statistically highly significant ($p<0.001$), while the difference between light to moderate smokers and heavy smokers was not ($p=0.581$). In our study, the analysis of Cd-B with regard to smoking habits clearly showed considerable difference between young healthy male non-smokers and smokers. In addition, the difference between light to moderate and heavy smokers was present, indicating the dose effect gradient.

Such results are consistent with the levels reported by many other studies of occupationally unexposed population. Staessen and associates (1990) have studied the cadmium burden in civil servant population in London. In this study, a similar mean Cd-B in the group of non-smokers was found as in our research, with the median 0.7 μg/L and the range of levels from 0.4 to 8.5 μg/L. They detected a higher mean level of Cd-B in smokers i.e. 1.5 μg/L as well. A similar mean Cd-B, as in our country, was also observed in Germany in a study of the influence of active and passive smoking on cadmium blood levels. The mean Cd-B observed in non-smokers was 0.85 μg/L, in passive smokers 0.93 μg/L, while in the group of smokers, a lower Cd-B was found than in our study (0.97 μg/L) (Shaham et al. 1996).

The review of mean Cd-B with regard to smoking habits showed considerable differences between individual groups. The Cd-B appeared to depend on smoking habits.

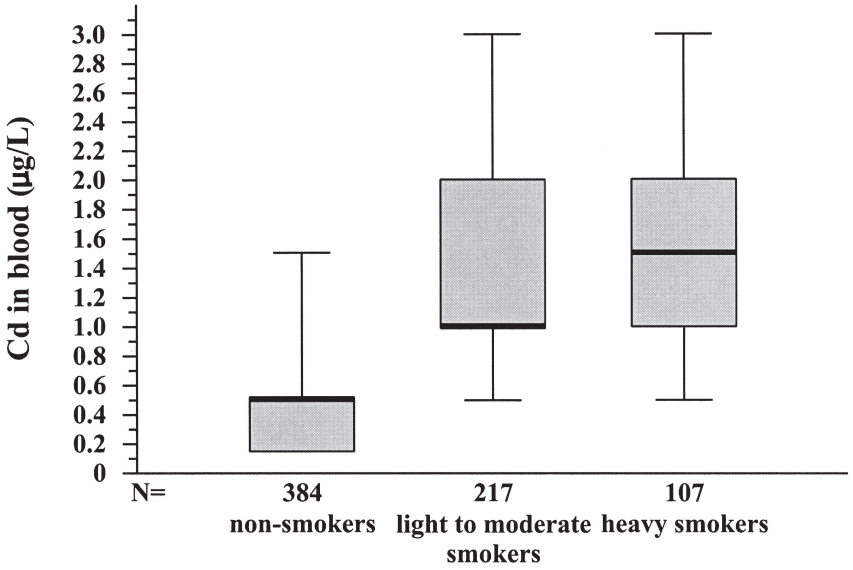

Figure 1. The median, interquartile range, 10th and 90th percentile values of Cd-B in three groups of male recruits from Slovenia regarding their smoking status. The box height represents the interquartile range, the whiskers the values of the 10th and the 90th percentile, and the thick line across the box represents the median value.

We could observe an increasing Cd-B gradient in non-smokers, light to moderate smokers and heavy smokers. Some other studies also reported similar findings (Satarug and Moore 2002; Hoffmann et al. 2001; Seifert et al. 2000; Maranelli et al. 1990).

The groups differed in smoking habits; our study, however, only considered the number of cigarettes smoked per day. The methodology employed in our research did not enable an analysis of the influence of inhaling on cadmium intake. Moreau et al. (1983), however, studied the influence, and the cadmium intake through smoking has proved important especially when the cigarette smoke is inhaled. Our research also did not inquire after the cigarette brands smoked. Due to this fact, the established degree of correlation between the cadmium blood content and the number of smoked cigarettes is perhaps smaller than the actual one: cigarette brands differ as to their cadmium content, which probably influences the quantity of cadmium intake through smoking.

Between the study groups, the differences proved to be statistically significant only in the case of non-smokers as compared to smokers. The differences in the cadmium blood levels between the individual groups of smokers did not prove to be statistically significant, although we could observe an increasing Cd-B gradient parallel to the growing number of smoked cigarettes.

Table 1. Frequency distributions of blood cadmium (Cd-B) level in military recruits in Slovenia related to smoking habits.

	Smoking status		
Cd-B level (μg/L)	**Non-smokers (%)**	**Light to moderate smokers (%)**	**Heavy smokers (%)**
	n=384	n=217	n=107
Below LOQ*	43.8	7.4	1.9
0.5-0.99	32.0	15.7	17.8
1.0-1.49	10.9	27.2	24.3
1.5-1.99	5.2	13.4	21.5
2.0-2.49	2.6	15.2	15.0
2.5-2.99	1.6	6.5	9.3
3.0 +	3.9	14.7	10.3

*LOQ - Limit of quantification < 0.49 μg/L

The absence of a statistically significant difference was probably the consequence of the fact that we could not consider the above mentioned influence of smoke inhalation or cigarette brands, but it could also be the result of the fact that indications of the number of smoked cigarettes were quite vague in the group of light smokers, and the smoking habits were not stable in younger years, respectively.

In addition to all these factors, the Cd-B was probably influenced by the number of recently smoked cigarettes, i.e. immediately before the blood sample taking. The cadmium absorption through the lungs is more readily than through the digestive tract (Satarug and Moore 2002; EHC 1992). It is thus possible that the recent cadmium exposure resulting from cigarette smoke inhalation has a considerable influence upon the Cd-B.

The findings of research confirm that the contribution of cigarette smoking to non-dietary cadmium intake is significant. It can be clearly observed that, besides the dietary intake, cigarette smoking is the most important source of cadmium in unpolluted environment. The contribution of smoking to total cadmium intake seems to be even greater than that of dietary intake. The influence of cigarette smoking on cadmium intake is further supported by the fact that there is an increasing Cd-B gradient in non-smokers, light to moderate smokers and heavy smokers. We think that more accurate information on the number of smoked cigarettes and the cigarette brand i.e. the cadmium content of tobacco would enable much better assessment of the influence of smoking on Cd-B.

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