Blood Lead, Cadmium and Zinc Correlations in Elderly Rural Residents

Pavlina L. Gidikova
Section of Hygiene and Medical Ecology, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria

Background: Combined exposure to heavy metals could be expected among residents of two villages in Stara Zagora Municipality due to significant pollution sources in the region, confirmed by increased heavy metal concentrations in plant samples identified by previous studies. The risk is increased for retired villagers who consume food produced in their own farms.

Aims: To determine blood levels of lead, cadmium and zinc and the correlation between them in high-risk elderly rural inhabitants in comparison with a control group.

Materials and methods: Lead, cadmium and zinc concentrations in whole venous blood were measured by atomic absorption spectrometry in exposed and in control groups adjusted by sex, age, smoking habit, lifestyle and duration of residence in the settlements.

Results: Blood values of lead and cadmium in the exposed group were significantly higher than these in the control group (p<0.001). A statistically significant positive correlation between lead and cadmium blood levels was estimated for the exposed group (ρ=0.39, p=0.023). Blood zinc levels correlated negatively with both lead (ρ=-0.41, p=0.015) and cadmium blood levels (ρ=-0.44, p=0.009). No correlations between the studied metals were found in the control group.

Conclusion: The observed results could be explained by a long-term combined exposure to lead and cadmium in the studied elderly residents. The negative correlation of zinc blood levels versus lead and cadmium could be result of competition. Complete protein intake and supplementation with zinc, calcium and iron after control measurements are advisable for elderly rural inhabitants to reduce the health risk from heavy metal exposure.
metal production and processing plants, a highway and several major roads and transport junctions. Although atmospheric levels of heavy metals are currently low, these pollutants are persistent in the environment, especially in soil, and are subject to bioaccumulation in food chains. In previous studies on seven oligoelements (lead, cadmium, nickel, zinc, arsenic, chromium, copper, manganese, and iron) in plant samples from the region the highest rates of concentrations above the limits\textsuperscript{10} were recorded for lead and cadmium in the mentioned two villages.\textsuperscript{11} The general population is exposed to lead and cadmium mainly via food consumption\textsuperscript{3-7}, hence the potential risk of cumulative exposure if contaminated food is ingested for a long period. The risk is especially high for retired village residents with low incomes, who usually consume food (vegetables, fruits, legumes, poultry and eggs) produced in their own farms. Presence of increased lead and cadmium in some plant samples implies a combined exposure and possible raised body burden levels of these metals.\textsuperscript{11} The blood concentrations of heavy metals may be used as reasonably good indicators for body burden if environmental exposure is stable\textsuperscript{3,6,9,12,13} Comparable persistence in the environment, bioaccumulation and inclusion in the food chain of lead and cadmium suggest a possible correlation between blood lead and blood cadmium concentrations after long-term exposure to both metals. On the other hand, competitive interactions involving zinc and excessive concentrations of ions with similar physicochemical properties (e.g. cadmium) can restrict the uptake and intestinal transport of zinc and thus its absorption and blood concentration.\textsuperscript{7}

The aim of this study was to determine blood levels of lead (BPb), cadmium (BCd) and zinc (BZn) in non-occupationally exposed elderly rural inhabitants and the correlation between them.

**MATERIALS AND METHODS**

Levels of lead, cadmium and zinc in whole blood were examined in 34 residents (4% of the total population) from the exposed villages of Zmeyovo and Borilovo (called hereafter exposed group), and in 16 residents (15% of the population) from the village Kolyo Marinovo situated far from pollution sources (control group). All three studied villages lie in Sredna Gora Mountain, but Zmeyovo and Borilovo are located near Stara Zagora town, while the control village is at the top of the mountain. The population, lifestyle, socioeconomic status and livelihood are very similar in the studied villages. The groups of donors were adjusted by sex, age and years of residence in the settlements. Each group consisted of equal numbers of women and men. There was no significant difference in the mean age of donors: 65.9±10.5 years for Zmeyovo and Borilovo villagers, and 67.7±8.5 years for the control group from Kolyo Marinovo. All donors were selected to be non-smokers to avoid the significant contribution of smoking to the intake of heavy metals reported by many authors.\textsuperscript{4,13,14} The participating subjects had been permanent residents of the studied villages for at least 10 years.

To assess heavy metal concentrations, 5 mL of venous blood were taken from the cubital vein of each subject in 6 mL vacutainers with K\textsubscript{3}EDTA. Venipunctures of all donors were made by the same experienced medical technician using needles and vacutainers from the same package. Each vacutainer was labeled with a code number to provide blind measurement of heavy metal concentrations. Written informed consents were obtained from all blood donors. The sampling procedure was approved by the authorized Ethics Commission. The heavy metal concentrations in whole blood were measured after microwave digestion in Multiwave 3000 using atomic absorption spectrometer 800 Analyst AAS (Perkin Elmer), operating with flame or graphite tubes with an additional injection hydride system. To avoid exogenous contamination all laboratory vessels were washed with 1M nitric acid and then thoroughly rinsed with deionized water. The methodologies for each metal were validated and monitored. The accuracy of the analytical procedure was controlled by measurement of lyophilized whole blood control materials (SERO, Norway). Reproducibility of investigation was verified with ten measurements of a random sample. The estimated relative standard deviations were 2.3% for lead, 2.8% for cadmium and 2.3% for zinc.

Mann-Whitney U-test to determine the differences between compared groups and Spearman correlation analyses using levels of lead, cadmium and zinc in blood were estimated by Statsoft Statistica v. 10. Percentage of donors with blood lead and cadmium concentrations exceeding the critical levels was determined. For BPb 20 μg/dL was regarded as a threshold for low-level lead effect in adults.\textsuperscript{7,15} Long-term exposure to cadmium with a blood concentration over 2 μg/L could lead to kidney damages with renal tubular dysfunction.\textsuperscript{16,17} This level of BCd was accepted as critical. For zinc...
the rate of donors with whole blood concentration under the lowest reference value of 440 μg/dL was determined.\textsuperscript{18}

RESULTS

Median values, first and third quartile (\(Q_1\)-\(Q_3\)) of lead, cadmium and zinc in blood for exposed and control groups are presented in Table 1. The range of values in the exposed group was very extensive – from minimum 4.64 to maximum 125.7 μg/dL. Non-parametric Mann-Whitney test showed significantly higher blood lead levels in the exposed group (\(p < 0.001\)). Furthermore, the first quartile in the exposed group was higher than the third quartile in the control. In 53\% of exposed donors (10 females and 8 males, most of them residing in the village of Zmeyovo) blood lead levels exceeded the 20 μg/dL, recommended as threshold for non-occupationally exposed adults. Moreover, in six women and five men, BPbs exceeded 40 μg/dL. In comparison, the range in the control group was from 6.04 to 24.92 μg/dL and only in one man and one woman did BPbs exceed slightly the threshold of 20 μg/dL.

Blood cadmium levels of the exposed group were also significantly higher in comparison with the control group (\(p < 0.001\)). Although the median value didn’t exceed the critical level, in thirteen villagers from Zmeyovo and one from Borilovo (41\% of all exposed) BCd concentrations were over 2 μg/L. The range of BCd in the exposed group (0.17 - 7.05 μg/L) was also wider than the range in the control group (0.16 - 2.06 μg/L).

Blood zinc levels in the exposed and control groups did not differ significantly. But in 14 (41\%) of the exposed subjects zinc concentrations in whole blood were under the lowest reference limit of 440 μg/dL versus no one from the control group. In 13 out of the 14 donors with BZn lower than 440 μg/dL blood lead concentrations were over the threshold limit of 20 μg/dL and cadmium in blood also exceeded the critical level of 2 μg/L. Only one person from these 14 had a normal BPb and BCd levels, but in this case BZn concentration was nearest to the reference value - 421 μg/dL.

No statistically significant difference was found in blood levels of the investigated heavy metals between men and women.

Correlation between blood levels of the three studied heavy metals were estimated using Spearman rank order correlation. Statistically significant positive correlation (\(\rho = 0.39, p = 0.023\)) was found between blood lead and blood cadmium levels. Statistically significant negative correlations were found between lead and zinc (\(\rho = -0.41, p = 0.015\)), and between cadmium and zinc in blood (\(\rho = -0.44, p = 0.009\)). These significant associations were observed in the exposed group only, but in the control group no significant correlation was found.

DISCUSSION

The significantly higher blood levels measured in the villagers of Zmeyovo and Borilovo (Table 1) and the positive correlation between BPb and BCd confirmed increased risk of simultaneous environ-
mental exposure to lead and cadmium in the region. The health hazard is even higher for the inhabitants with BPb over 20 μg/dL - 7 people and over 40 μg/dL - 11 people. At these levels biomarkers for adverse effects of lead might be observed like erythrocyte protoporphyrin elevation, increased urinary δ-aminolevulinic acid and elevated coproporphyrin, and at the higher concentrations - anemia, gastrointestinal and neurological disturbances. In 14 of the villagers with BPb over 20 μg/dL concentration of cadmium in blood also exceeded the critical level of 2 μg/L. Above this BCd level after long-term exposure accumulation in the kidney and tubular dysfunction might be observed, manifested with increased urinary N-acetyl-β-D-glucosaminidase and β₂-microglobulin. An increased risk of osteoporosis should be taken into consideration because of the combination of cadmium exposure and elderly age. Question arises about a possible synergistic interaction at simultaneous exposure to cadmium and lead. Interaction profile for joint toxic action of lead and cadmium estimated greater than additive adverse effect of mixtures only for the reproductive (testicular) function. Neurological effects were greater than additive for cadmium on lead joint toxic action with low confidence. Renal, cardiovascular and hematological adverse effects of cadmium and lead combined influence were determined as additive.

Increased blood levels of lead and cadmium registered in the elderly inhabitants of Zmeyovo and Borilovo could be due to the persistence of these heavy metals in the environment, incorporation into the food-chain, and permanent intake via ingestion of plant and animal foods produced locally. Bioaccumulation typical for both metals results in chronic endogenous exposure through reentrance in the blood from tissue stores, especially in cases of acid-base imbalance, oxidative stress and other homeostatic disturbances. Osteoporosis inherent of elderly could result in re-releasing of stored lead from bones into the blood. This way, lead exposure that occurred decades earlier may be a source of ill-health in the elderly. Additional cause of high BPb and BCd could be a low protein diet and deficiencies of calcium, iron and vitamin C, because each of these disorders might increase absorption, retention and accumulation of lead and cadmium. Such dietary deficiencies are not exceptions for retired rural people with low income. Ensuring sufficient intake of complete protein and, if necessary, calcium and ferrous supplements are advisable for reducing the health risk from heavy metal exposure in this population.

An analysis of individual levels showed that in 41% from the exposed (13 villagers from Zmeyovo and 1 from Borilovo) zinc levels in whole blood were under the lowest reference limit, almost all coinciding with increased levels of BPb and BCd. Very likely the main reason for zinc insufficiency is inadequate nutrition of the elderly rural population. Typical diet of rural retirees includes bread, legumes, fruits, vegetables, which are rich in phytate and hemicelluloses that could decrease zinc absorption. Because of their low income retirees usually consume less than the required amounts of meat, fish and seafood which are essential sources of zinc and protein. Other studies determined insufficient zinc intake and absorption among the rural population because of typical diets. An US National Health and Nutrition Examination Survey found that 35-45% of adults aged 60 years or older had zinc intakes below the estimated average requirement, and even taking into account both food and dietary supplements 20-25% of older adults still had inadequate zinc intake. On the other hand, the significant negative correlations of zinc levels with both BPb and BCd observed in this study suggest a possible additional inhibition of zinc absorption in the village residents exposed to lead and cadmium. Significant negative correlations were found in the exposed group, but not in the control, thus confirming the association of increased lead and cadmium blood levels with zinc deficiency.

The health risk for elderly rural inhabitants exposed to lead and cadmium could result not only from their specific adverse effects, but also from superimposed zinc insufficiency. Cadmium could actually displace zinc in some of its important enzymatic and organ functions, interfering with these functions or preventing them from being completed. Studies of the general population have investigated the potential relationship between zinc intake and blood lead. Ingestion of a zinc sulfate supplement from men and women with adequate dietary zinc, copper and iron intake did not significantly affect blood lead levels or lead balance. The results suggest that supplemental zinc at the recommended level does not affect the bioavailability of lead in humans already receiving adequate zinc. But the effects of BPb and BCd in a situation of inadequate zinc intake are not currently clear. Evaluation of joint toxic action for neurological effects has determined less
than additive effect of zinc on lead. Moreover, the potential adverse hematological effects of lead are likely to be lowered in mixture with zinc. Intake of zinc supplements probably would not reduce the absorption of lead and cadmium, but could prevent the adverse effects of zinc insufficiency.

Blood lead levels in the exposed group were much higher than registered in studies performed on different continents for environmentally exposed adults: in Mexico - 11.9 μg/dL, in China 13.2 μg/dL for men and 10.1 μg/dL for women, in the United States - 6.2 μg/dL for men with mean age 67 years, and in Italy – 4.51 μg/dL for men and 3.06 μg/dL for women. Blood cadmium levels in the exposed group was also higher in comparison with reported for non-smokers in Singapore - 0.21 μg/L for men and 0.26 μg/L for women, Morocco 1.1±0.8 μg/L for men and 0.8±0.4 μg/L for women, and in Swedish twins - 0.41 μg/L. An investigation of reference intervals for blood Cd and Pb in the general population of Sardinia (Italy) showed levels much lower than ours - BPb (4.12 μg/dL) and BCd (0.56 μg/L) geometric mean for sample population over 60 years of age. These comparisons confirm the increased risk of exposure to lead and cadmium in the studied elderly rural population.

A limitation of this study is that investigations were performed in only two villages. It would be better to study a representative sample from Stara Zagora town encompassing different age groups. The investigations of blood lead and cadmium levels in children are very important, because adverse health effects in children could be manifested at much lower body burdens.

CONCLUSION

The increased blood levels of lead and cadmium and the significant positive correlation between them supposed a long-term combined environmental exposure of elderly inhabitants in the region. The negative correlation of zinc blood levels versus lead and cadmium could be a result of competition of similar ions for absorption and retention.

Ensuring sufficient intake of complete protein and, if necessary, calcium and ferrous supplements are advisable for reducing the health risk from heavy metal exposure in elderly rural inhabitants. Zinc supplementation could be useful only after individual control blood test.

ACKNOWLEDGEMENTS

This study was supported by a grant NIP 13/2015 from the Faculty of Medicine, Trakia University, Bulgaria. The author would like to express gratitude to all donors who participated in the investigation, to Assoc. Prof. Rositsa Deliradeva for the support, and to Assist. Prof. Gergana Sandeva for the English proofreading and editing.

REFERENCES

1. World Health Organization. Health risks of heavy metals from long-range transboundary air pollution. Copenhagen: WHO; 2007.

2. Jaishankar M, Tseten T, Anbalagan N, et al. Toxicity, mechanism and health effects of some heavy metals. Interdiscip Toxicol 2014;7(2):60-72.

3. World Health Organization. Air Quality Guidelines for Europe. 2nd ed. Copenhagen: WHO; 2000.

4. Järup L. Hazards of heavy metal contamination. Br Med Bull 2003:68:167-82.

5. De Winter-Sorkina R, Bakker MI, van Donkersgoed G, et al. Dietary intake of heavy metals (cadmium, lead and mercury) by the Dutch population. Bilthoven: RIVM; 2003.

6. Pizzol M, Thomsen M, Andersen MS. Long-term human exposure to lead from different media and intake pathways. Sci Total Environ 2010;408(22):5478-88.

7. World Health Organization. Trace elements in human nutrition and health. Geneva: WHO; 1996.

8. George PM, Walmsley TA. Handbook of Trace Metal Analyses for Health. 4th ed. New Zealand: Canterbury Health Laboratories, Christchurch; 1998.

9. Roney N, Colman J. Interaction profile for: lead, manganese, zinc, and copper. Atlanta, U.S: Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry; 2004.

10. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union, December 20, 2006;L 364:5-24.

11. Gradeva H, Sandeva G, Gidikova P, et al. Heavy metals in plants, soil and water from Stara Zagora region: defining locations for population exposure measurements. Trakia Journal of Sciences 2012;10(3):125-9.

12. Björkman L, Vahter M, Pedersen NL. Both the environment and genes are important for concentrations of cadmium and lead in blood. Environ Health Perspect 2000;108(8):719-22.

13. Černá M, Pěváčková V, Beneš B, et al. Reference values for lead and cadmium in blood of Czech population. Int J Occup Med Environ Health 2001;14(2):189-92.
14. Apostoli P, Baj A, Bavazzano P, et al. Blood lead reference values: the results of an Italian polycentric study. Sci Total Enviro 2002;287(1-2):1-11.
15. Bull S. HPA Compendium of Chemical Hazards – Lead. Version 2. HPA, UK; 2011.
16. Agency for Toxic Substances and Disease Registry. Case Studies in Environmental Medicine (CSEM) - Cadmium Toxicity. The Agency, Atlanta, USA; 2008.
17. Bull S. HPA Compendium of Chemical Hazards – Cadmium. Version 5. HPA, UK; 2011.
18. ARUP Laboratories. Zinc Quantitative, Whole Blood; 2016. Available from: http://ltd.aruplab.com/Tests/Pub/2009373.
19. Gulson BL, Mahaffey KR, Mizon KJ, et al. Contribution of tissue lead to blood lead in adult female subjects based on stable lead isotope methods. J Lab Clin Med 1995;125(6):703-12.
20. Nordberg GF, Nogawa K, Nordberg M. Cadmium. In: Nordberg GF, Fowler BA, Nordberg M, editors. Handbook on the Toxicology of Metals. London: Elsevier; 2015:668-716.
21. Garrido Latorre F, Hernandez-Avila M, Tamayo Orozco J, et al. Relationship of blood and bone lead to menopause and bone mineral density among middle-age women in Mexico City. Environ Health Perspect 2003;111:631-6.
22. Hernández-Avila M, Smith D, Meneses F, et al. The influence of bone and blood lead on plasma lead levels in environmentally exposed adults. Environ Health Perspect 1998;106(8):473-7.
23. Cheng Y, Willett WC, Schwartz J, et al. Relation of nutrition to bone lead and blood lead levels in middle-aged to elderly men. Am J Epidemiol 2001;147(12):1162-74.
24. King JC, Turnlund JR. Human zinc requirement. In: Mills CF, editor. Zinc in human biology. Berlin: Springer-Verlag; 1989:335-50.
25. Ervin RB, Kennedy-Stephenson J. Mineral intakes of elderly adult supplement and non-supplement users in the third national health and nutrition examination survey. J Nutr 2002;132:3422-7.
26. Kies C, Ip SW. Lead bioavailability to humans from diets containing constant amounts of lead: Impact of supplemental copper, zinc and iron. Trace Subst Environ Health 1990;24:177-84.
27. Chen C, Wang X, Chen D, et al. Tofu consumption and blood lead levels in young Chinese adults. Am J Epidemiol 2001;153(12):1206-12.
28. Chia SE, Chan OY, Sam CT, et al. Blood cadmium levels in non-occupationally exposed adult subjects in Singapore. Sci Total Environ 1994;145(1-2):119-23.
29. Khassouani CE, Soulaimani R, Maura S, et al. Blood cadmium concentration in the population of the Rabat area, Morocco. Clinica Chimica Acta 2000;302(1-2):155-60.
30. Forte G, Madeddu R, Tolu P, et al. Reference intervals for blood Cd and Pb in the general population of Sardinia (Italy). Int J Hyg Environ Heal 2011;214(2):102-9.
Корреляция содержания свинца, кадмия и цинка в крови у пожилых жителей сельских районов

Павлина Л. Гидикова

Секция гигиены и медицинской экологии, Медицинский факультет, Фракийский университет, Стара Загора, Болгария.

Адрес для корреспонденции:
Павлина Гидикова, Секция гигиены и медицинской экологии, Медицинский факультет, Фракийский университет, ул „Армейска“ № 11, 6000, Стара Загора, Болгария.
E-mail: pggidikova@yahoo.com
Tel: +359 42664329

Дата получения: 06 марта 2017
Дата приемки: 16 августа 2018
Дата онлайн публикации: 18 августа 2018
Дата публикации: 31 марта 2019

Ключевые слова: lead, cadmium, zinc, elderly rural population

Введение: Комбинированное воздействие тяжелых металлов можно ожидать среди жителей двух сёл в муниципалитете Стара Загора из-за значительных источников загрязнения в этом районе, что подтверждается повышенными концентрациями тяжёлых металлов в образцах растений, выявленных в предыдущих исследованиях. Существует более высокий риск для пенсионеров из этих сёл, которые потребляют продукты питания, выращенные на их собственных фермах.

Цели: Определить уровень содержания свинца, кадмия и цинка в крови и корреляцию между ними у пожилых жителей сельских районов с высоким риском по сравнению с контрольной группой.

Материалы и методы: Концентрации свинца, кадмия и цинка в цельной венозной крови измеряли с помощью атомно-абсорбционной спектрометрии экспонированных лиц и контрольных групп, которые были разделены по полу, возрасту, курению, образу жизни и продолжительности проживания в населённых районах.

Результаты: Концентрации свинца и кадмия в экспонированной группе были значительно выше, чем в контрольной группе (р <0.001). Статистически значимая положительная корреляция между уровнями кадмия и свинца в крови была установлена в группе экспонированных лиц (ρ = 0.39, р = 0.023). Уровни цинка в крови отрицательно коррелировали как с уровнем свинца (ρ = -0.41, р = 0.015), так и уровня кадмия в крови (р = -0.44, р = 0.009). В контрольной группе не установлена корреляция тестируемых металлов.

Вывод: Наблюдаемые результаты могут быть объяснены длительным комбинированным воздействием свинца и кадмия в исследуемой популяции пожилых людей. Отрицательная корреляция между уровнями цинка в крови и свинца и кадмия может быть связана с конкуренцией. Принятие полноценных белков и добавок цинка, кальция и железа после контрольных измерений рекомендуется для пожилых людей в сельских районах, чтобы снизить риск для здоровья от воздействия тяжёлых металлов.