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Human Biomonitoring of Trace Elements (Zn, Cu, Mn, Cd and As) Level in a Healthy Urban Population in Türkiye

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ABSTRACT: Human biomonitoring (HBM) studies are carried out to examine the effects of environmental pollutants on human health and to identify risks to public health, with the increasing awareness of environmental pollution around the world. The aim of our study is to determine the level of trace elements and to perform preliminary human biomonitoring of a healthy urban population in Türkiye. For this purpose, healthy individuals living in the same region and not known to have occupational or any other exposures were included in the study. Element levels from serum/blood of volunteers were determined. In this study, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to analyze in order to determine the multi elements in a more precise way with an improved analytical quality of results. According to our study's results, Mn and As ($p<0.05$) were found statistically significant with age. Smoking had an effect on Cd levels ($p<0.01$) and there were significant differences between males and females for only Zn ($p<0.05$). The results of our study provided more information on the reference values of Zn, Cu, Mn, Cd and As elements of the Turkish population and reported the influences of age, gender and smoking habits on trace element profiles.

KEYWORDS: Biomonitoring; trace elements; ICP-MS; blood; serum.

1. INTRODUCTION

People are exposed to trace elements naturally occurring in the earth's crust and from many sources such as environmental and industrial workplaces. Some trace elements such as copper, manganese, chromium, molybdenum, selenium, iron, zinc, and sulfur are essential for the human body as they play a catalytic role in biochemical processes. However, in the case of high concentration exposure, toxic effects can be seen in the human body. Elements that have a toxic effect such as arsenic (As), cadmium (Cd), lead (Pb), nickel (Ni) should not be present in the body even in low doses. The main threats to human health from heavy metals are associated with exposure to Pb, Cd, Ni and As. Toxic metals exert a strong toxic effect by disrupting cellular events such as growth, reproduction, differentiation, damage repair processes and apoptosis. The toxicological potential of a heavy metal depends on the heavy metal type, chemical form, dose, route of exposure, age, sex, genetic and nutritional status of the individual [1-3].

Heavy metals are well-known important environmental pollutants characterized by their toxicity in living organisms. People can be exposed to metals that cause pollution by mixing with different environmental elements (water, soil and air) in a variety of ways, such as through the skin, respiratory tract or drinking water and food contaminated with heavy metals. Since metals have a long half-life and are not metabolized in the body, they cause bioaccumulation. This accumulation creates serious health risks for humans in cases of chronic exposure and different toxic effects on various body tissues and organs [2, 3].

Trace elements, systemic toxicants that initiate cascades of organ dysfunction in humans, even at low levels in the body are one of the common problems requiring worldwide attention. Researchers have shown that heavy metal exposure causes DNA damage and various carcinogens [4-7]. Each heavy metal has its own

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unique mechanical process where it exhibits toxicity. The main mechanisms by which most heavy metals cause toxicity include the formation of reactive oxygen species (ROS), the inhibition of enzyme activities, and the weakening of antioxidant defense systems. Heavy metals have been studied extensively by international organizations such as the World Health Organization (WHO), and their health effects have been regularly reviewed and monitored. According to the International Agency for Research on Cancer (IARC), these metals are classified as "known" or "possible" human carcinogens based on epidemiological and experimental studies [2, 5]. Despite the fact that heavy metals have long been known to have adverse health effects, people continue to be exposed to them due to environmental pollution, mining and smelting processes, industrial production and use, domestic and agricultural use, as well as occupational exposure.

With the increasing awareness of environmental pollution around the world, human biomonitoring (HBM) studies are conducted around the world to monitor and study the effects of environmental pollutants on human health. These studies are important for assessing chronic exposure and determining exposure levels to environmental pollutants in the general population, population subgroups, and individuals. Metal concentrations in human fluids often reliably reveal undesirable environmental exposures and are therefore often tested by human biomonitoring studies [9-21]. In most of the studies conducted so far, occupational exposure has been demonstrated and investigated, or populations living in industrial areas with significant environmental pollution have been studied

The aim of this study is to conduct a preliminary biomonitoring investigation by determining the trace elements levels of healthy individuals living in the same area without intense environmental pollutants in Türkiye

2. RESULTS

The study population included 38 healthy individuals (16 male, 22 female) without occupational or any other exposure in the age range of 38.85 ± 6.7. With regard to smoking status, 18 (47.37%) of the participants had a light smoking habit and 20 (52.63 %) were nonsmokers. The mean and SD values of 5 different trace elements (Zn, Cu, Mn and As, Cd) in individual's blood or serum of the elements are presented in Table 1.

Table 1. Trace element levels (Mean ± SD) in serum or blood of participants.

Elements (µg/L) N:38	Specimen	(Mean ± SD)
Mn	Blood	10.10 ± 22.04
Cu	Serum	800.66 ± 139.85
Zn	Serum	1148.93 ± 287.97
As	Serum	3.17 ± 2.74
Cd	Blood	1.18 ±1.35

Association between trace elements levels and gender, smoking and age are demonstrated in Table 2 with the relevant statistical parameters. Cd were found to be statistically significant differences between smokers and nonsmokers ($p<0.01$). Only zinc was statistically significant in males compared to females, and other trace elements were not statistically significant with regard to gender ($p<0.05$). When participants were divided into groups of under 40 years and over 40 years of age, Mn and As ($p<0.05$) were found to be statistically significant.

Table 2. Association between trace element's levels and gender, smoking, age

Elements (µg/L)	Age (mean ± SD) N			Smoking status (mean ± SD) N			Gender (mean ± SD) N		
	>40 (n=18)	<40 (n=20)	p	smoker (n=18)	non-smoker (n=20)	P	male (n=16)	female (n =22)	P
Mn	14.64 ±29.35	6.01± 12.92	**<0.05**	13.88±31.70	7.35±12.30	>0.5	2.14±1.12	15.89±28.04	>0.5
Cu	814.65±165.19	788.01±115.00	>0.5	814.85±116.50	787.83 ± 159.87	>0.5	795.36±150.22	804.58±134.99	>0.5
Zn	1192.50±269.17	1109.52±305.06	>0.5	1101.45±304.34	1191.90 ±272.51	>0.5	1261.94±324.60	1065.41±230.67	**<0.05**
As	4.22±3.45	2.20±1.40	**<0.05**	3.21±2.08	3.13±3.24	>0.5	3.02±3.59	3.30±1.76	>0.5
Cd	2.01±1.42	1.65±1.10	>0.5	1.73±1.65	0.61±0.35	**<0.01**	1.13 ±0.79	1.29±1.36	>0.5

3. DISCUSSION

Data on the reference or range values of a wide range of trace elements in whole blood plasma or serum from populations provide important information to indicate potential exposures of populations, their nutritional status, clinical monitoring, and in the evaluation of forensic cases. Due to increased exposure to environmental factors (food, air, water) and trace elements, various societies have conducted studies at specific intervals and regions. Many studies have been done in areas with heavy industrial environmental pollution or studies that show occupational exposure. However, with increasing awareness of environmental pollution, the population studies have also varied. In particular, recent studies include data on the analysis of various trace elements in biological fluids (blood, serum, urine) of the healthy population. [14,26]. Studies involving occupational exposure to heavy metals have been reported previously in the Turkish population [6, 7]. Data on healthy individuals are very limited in these studies.

Our study provides reference information on the mean values of 5 trace elements in the whole blood/serum of healthy individuals without occupational exposure in the Turkish population. The (mean ± SD) levels for Zn, Cu, Mn and, As and Cd were 1148.93 ± 287.97; 800.66 ± 139.85; 10.10 ± 22.04; 3.17 ± 2.74; 1.18 ±1.35 respectively. According to our study's results, trace elements were in the range of the reference values in participants.

In this study, the mean value of Zn concentration were found to be 1148.93 ± 287.97 and it was significantly higher in male participants compared to females ($p<0.05$). The levels of Zn were similar to those of the Austrian population (1150 µg/L, as per Komarova et al.) [23]. While it was relatively higher than the German population (1020 µg/L as per Heitland, and Koster 2006) [24], Serbiaian (571µg/L as per Stojsavljević et al.) [25], Korean population (872.7 µg/L as per Kim et al.) [26] and higher than the Italian population (6418 µg/L as per Bocca et al.) [27]. Only a few studies have reported serum Zn concentrations for gender, and our results were consistent with previous studies in different populations such as 739 µg/L for males and 700 µg/L for females in the Brazilian population [28], 902.4 µg/L for maless and 884.1 µg/L for females in Korea [26],There are studies suggesting that smoking affects Zn levels and is highly correlated with low zinc levels [27]. However, there was no significant difference between Zn levels, smoking, and age in our study.

The mean serum Cu concentrations were found to be 800.66±139.85 and showed a general similarity with the literature [25, 26, 29]. The gender and smoking related difference in Cu blood concentration was reported in previous studies. Nevertheless, smoking, gender, and age did not appear to be significant factors in our study [26, 27].

Our data showed mean blood Mn value as 10.10 ± 22.04 µg/L, which is similar to the level of 10.8 and 11.1 µg/L, measured at the Korean national survey [26, 30]. Canadian levels were 10.8 µg/ L, and slightly higher than those reported for populations in Italy 8.9 µg/L (Bocca et al.) [27]. Brazil 9.6 µg/L [31]. However, our value was lower in comparison to Australian 17.3 [23] and Serbian populations 14.5 [25]. The results of Komarova et al demonstrated the effect of age on Mn levels (>40 age group 10.2; <40 age group 9.5) and are consistent with our study (>40 age 14.64; <40 6.01); these were evaluated to be statistically significant ($p<0.05$). According to our studies, blood Mn was influenced by gender, as females (15.89±28.04) accumulated more Mn than males (2.14±1.12) which correlates with previous studies [27, 32, 33]. However, this result is not statistically significant ($p>0.05$). Previous studies showed that females have higher blood Mn than males. Blood Mn content was not affected by smoking in our study as shown in the literature.

While most women have more Cu and Mn in their bodies than men, males have more Zn. Zn plays important roles in men's health, such as aiding immune functions, maintaining healthy cell growth, maintaining prostate health, sexual health, and testosterone hormone levels. Moreover, high Zn concentrations have been reported in the prostate gland and semen. Because Zn plays a role in the antibacterial activity of seminal plasma, sperm production and viability, sperm membrane stabilization and prevention of spermatozoa degradation, preserving prostate health, sexual health and testosterone hormone levels. For this reason, it was stated that the Zn requirement of men was higher than that of women [34, 35]. It has been noted that males generally have lower Mn levels and generally higher Fe stores than females, and that increased Fe stores (ferritin levels) may be associated with decreased Mn absorption [27,36,37].

The mean serum Cd concentrations were found to be 1.18 ±1.35 µg/L, which is relatively different from other populations; it is higher than that reported for Chinese (0.78 as per Zhang, et al.) [38] , Germans 0.44 (Heitland, and Koster) [24], Italians (0.53 as per Bocca et al) [27], Brazilians, (0.4 as per Nunes et al.) [24], and Australians (0.8 as per Komarova et al.) [23]. Researchers have shown that the concentration of Cd in the blood increases with age, which is consistent with our results. We found that the cadmium level was higher for the >40 age group, but it was not statistically significant. This difference can be explained by the wider age range in previous studies compared to our study. In this study, a plateau level may be reached in individuals over 50 years of age, probably due to age-related deterioration in renal function. [39]. Also, in agreement with previous studies, blood Cd levels were higher in females than in males, but it was not statistically significant [39]. In our study, the Cd level of smokers was found to be higher than that in non-smokers ($p<0.01$). Previous reports and studies, which are specific to the Turkish population, have also confirmed smoking-induced blood Cd increase [41, 42]. Cigarette smoking is known to be an important source of Cd exposure. Each cigarette contains approximately 1 µg of this element, and since 25-35% of it is absorbed into the bloodstream, it significantly increases the amount of Cd in the body [43].

In the present study, the mean serum As concentrations were found to be 3.17 ± 2.74 µg/L. The concentrations of As in the serum was significantly different between the under 40 and over 40 age groups ($p<0.01$). These results are in accordance with studies reporting that arsenic increases with age [24, 26]. In arsenic biomonitoring studies, different biological samples such as urine, hair, nails and blood are used as biomarkers of human exposure. Authors cited plasma samples as potential matrices along with whole blood samples, although urine is the most suitable biological sample for arsenic. Arsenic in the blood is cleared within a few hours after absorption, but in populations that are constantly exposed to arsenic, for example in drinking water, blood concentrations reach a constant point and this is an indicator of chronic exposure.

Arsenic concentrations in blood or serum are much lower than those in urine, hair or nails, so low-level exposures are difficult to detect analytically [43, 45, 46]. There is no well-defined reference range or health-based threshold level for blood and serum in the literature, as there are not enough studies reporting arsenic concentrations in these samples. Studies have mostly included whole blood analysis as an indicator of arsenic, and serum results are quite limited. As a result, we were unable to compare our study results with those of other populations.

4. CONCLUSION

Human biomonitoring, which provides valuable information about monitoring environmental exposure and nutritional status contributes to determining the extent to which the population is affected by environmental pollutants, identifying potential health risks, and developing policies and programs to protect human health. For this reason, it is important to conduct biomonitoring studies involving various pollutants in the Turkish population. The present study has revealed significant differences of Zn between males and females, smoking related Cd, and the trend of increased As and Mn with age in individuals. Our study was carried out in a certain region and with a limited number of individuals. It is recommended that the future studies can be carried out in a larger area and with more participants.

5. MATERIALS AND METHODS

5.1. Study Subjects

In this study, consisting of 38 healthy individuals (16 males and 22 females; 38.85 ± 6.7 years; ranging from 25 to 53 years), were recruited and 18 of them were smokers and 20 were non-smokers. Informed consent was obtained from each individual randomly selected from the Turkish population. The volunteers also completed detailed questionnaires regarding factors that influence trace elements levels, such as smoking and occupational exposure. Additionally, none of the female volunteers were pregnant and a EDTA vacutainer glass tube was used for the collection of all blood samples in accordance with the Declaration of Helsinki. The study design was reviewed and approved by the Medipol University Faculty of Medicine Non-interventional Clinical Researches Ethics Committee in Istanbul, Türkiye (approval number:10840098-772.02 -E.5573).

5.2. Reagents

Ultrapure water with a resistance of 18 MΩ cm was obtained from a Milli–Q® purification device (Millipore Corporation, Bedford, MA, USA). Nitric acid (65 %) and tritonX100 was purchased from Merck (Darmstadt, Germany) and Sigma Aldrich (Schnelldorf, Germany) respectively. Element standard solutions of As, Cu, Mn, Cd, Zn were supplied by VWR (Darmstadt, Germany). Y Pure Single-Element Standard (Internal standard-IS) was purchased from Perkin Elmer (Shelton, CT, USA).

5.3. Instrumentation

Analyses were performed with a NexION 300X ICP-MS system (Perkin Elmer, Waltham, MA, USA) coupled to an SC FAST sample introduction system and an SC 2DX autosampler (Elemental Scientific, Omaha, NB, USA). The instrumental operating conditions are shown in the Table below (Table 3). Syngistix™ software was used to control and data acquisition.

Table 3. ICP-MS instrumental conditions

Parameter	Type/Value/Mode
Radiofrequency power/W	1600
Nebulizer	Glass concentric
Spray chamber	Glass cyclonic
Sample and skimmer cones	Nickel
Plasma gas flow (L/min)	18.0
Auxiliary gas flow (L/min)	1,2
Nebulizer gas flow (L/min)	0,9
Sample uptake rate (µL/min)	300
Number of replicates	3
Dwell time (ms)	50
Universal Cell Technology	KED mode

5.4. Sample Preparation

Samples were prepared according to previously published methods [19 ,22]. Briefly, 0.2 mL serum samples were diluted to 2.0 mL with 1% (v/v) HNO_3 and 0.4 mL of whole blood samples were diluted with 3.5 mL 0.65% (w/v) HNO_3 (triton 0.1%) in 15 mL falcon tubes after the addition of IS (20 µL) and mixed thoroughly on a vortex-mixer prior to ICP-MS analysis.

5.5. Statistical Analyses

Statistical analysis was performed using IBM SPSS release v.28.0 (IBM Corporation). The variables were investigated using visual methods (histograms, probability plots) and analytical methods (*Kolmogorov-Simirnov*) to determine whether or not they are normally distributed. Descriptive analyses were presented using means and standard deviations for normally distributed variables. Comparison of variables between groups was performed by *student's t-test* or the *Mann– Whitney U-test*. An overall %5 type-1 error level was used to infer statistical significance.

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