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Levels of toxic metals and trace elements in autopsy liver tissue samples

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Abstract
The aim of the study is to determine the levels of toxic metals (cadmium and lead) and trace elements (zinc and copper) in the liver tissues of autopsy cases in Ankara and to evaluate the statistical relationship of these metals with subjects' physical properties, smoking status, occupational exposure and environmental factors. Results are expected to establish the average burden of involved metals in liver tissue of Turkish population. Hepatic trace elements and toxic metal levels were assessed in 119 autopsy cases. Digestion procedure for liver tissues was carried out using a Mars Xpress microwave system (CEM, Matthews, USA) with PTFE microwave digestion vessels. Lead and cadmium were analyzed by Graphite Furnace Atomic Spectrometry; copper and zinc were analyzed by Flame Atomic Absorption Spectrometry. Average liver levels of metals and trace elements were found 29.5 µg/g, 216 µg/g, 0.39 µg/g and 4.38 µg/g dry weight for copper, zinc, lead and cadmium, respectively. Copper and lead levels were found higher in men. There was a significant decrease in copper levels with age. Lead levels in city dwellers were significantly higher and there was no correlation between metal levels and occupations of the individuals. Significant increase in copper, lead and cadmium were observed in smokers' liver tissues. All metal levels were compared with each other and positive correlation was found between zinc-copper, lead-zinc and copper-zinc levels ($p<0.01$). In Turkey, it is the first study which gives liver tissue levels of trace elements and heavy metals of autopsy samples in a city which has a median-scale industrial development.

Keywords: Liver tissue, autopsy, copper, zinc, lead, cadmium, AAS

Introduction

Heavy metals are natural elements of earth crust and widely found in air, water, soil and biological systems [1]. The exposure is commonly originated from engine exhaust, industrial activities, agricultural drugs, wastes etc [2]. Although some metals (iron, cobalt, copper, fluorine, iodine, selenium, copper, manganese, chromium) are essential trace elements, they are extremely toxic to living organisms at high-dose exposures [3]. Besides this fact; some naturally-occurring metals like arsenic, cadmium, lead, mercury, vanadium may be highly toxic to living organisms, even in low-dose concentrations [4-5].

Cadmium is naturally occurs element on earth and also by-product of zinc, lead and copper extractions in industrial process. Due to being mainly the part of wide variety of fertilizers and pesticides, it can directly spreads by soil [6]. In humans, exposure is generally seen especially from foods and cigarette smoking. Mushrooms, shellfishes, mussels, seaweed and cacao powder are the examples of some foods that have highly content of cadmium [7].

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Smoking has much more importance than the foods in cadmium exposure. Smokers can get twice amount of cadmium than that of non-smokers. Reference blood cadmium level was determined for smokers as 0.3-3.92mg/ but for non-smokers as 0.3-1.2mg/l [8].

Lead can be easily shaped and refound element that can constitute various kind of alloys with the other elements. These types of composites are generally used in pipes, rechargeable batteries, scales, bullets and armaments, cable coating and radiation protection layers. They are widely used in automobiles and the accumulators [9].

Environmental lead level has been increased 1000 times in the last three centuries. The most salient increase observed between 1950-2000 due to high consumption of the oils contains lead as a additive [10-11]

The 99 percent of absorbed lead binds to erythrocytes and stays 30-35 days in blood. Then it is transported to tissues like liver, kidney, aorta, brain and lungs in 4-6 weeks. No consequential effect can be seen the blood lead level for adults above 50 □g/dl and for children above 40 □g/dl. [12].

Trace elements are metals that are necessary for maintaining healthy physiological processes of the body.

They usually function as a part of a vital enzyme and play important roles in hormone regulations, neurotransmission mechanisms, metabolic pathways, and in elimination of toxins from the body [13-15]. These elements are naturally found in daily diet or commercially available. Heavy metals are stable natural constituents that cannot be metabolized or degraded by physiological routes. Therefore, they tend to accumulate in different body compartments like liver, kidney, brain, bone etc. [16]. This accumulation is the starting point of human toxicity due to exposure or overdose. Industrial sources and relevant environmental contamination is the major exposure type in many occupations and daily life [17].

Zinc can be found in water, air and almost every food. Elemental form is blue-white color and bright. The zinc powder can explode in high humidity media. Metallic zinc can be used in steel and iron coating by galvanization. Brass and bronze alloys also contain zinc [18]. Zinc compounds also foıund in vitamin, suntan oils, ointments and deodorants [19]. Zinc is essential trace element for body and generally taken by the foods [10]. It has role on metabolic pathways; protein, carbohydrate, energy, nucleic acids, lipids and heme synthesis, gene expression, tissue synthesis and embryogenesis [19].

Copper also a necessary trace element for humans and take part in heme synthesis [20]. It is found more or less in every tissues of the body. It contributes a lot of blood metabolism functions and serves as active ion parts of some enzymes. Human can take copper 1-2 mg daily and it is absorbed by intestine. Avarage serum concentration is 90µgr/100 ml. and 96 percent stays as bonded to ceruloplasmin. It is also found in cytochrome oxidase, catalase, peroxidase tyrosinase, monoamine oxidase and ascorbic acid [21]. The copper in the circulation is mostly distributed to liver tissue [22].

There are some protective mechanisms for tissues prevents from free radicals. They should be removed from the tissues by the help of enzymes like glutathione peroxidase or superoxide dismutase. Ceruloplasmin and metallothionein are also protects the cells [23].

Essential elements Cu, Mn, Se and Zn are the important parts of the protective enzymes. Zinc protects sulfhydryl groups against oxidation, inhibits the formation of reactive oxygens by transition metals [24].

Toxic metals by targeting the specific biochemical process or organelles to effects the multiple organ systems. Toxic effects of elements contains the interaction of free metal ion and specific target protein [25].

Most of the toxic elements, by targeting specific biochemical processes or organelles, effect multiple organ systems. Toxic effects of elements involves interactions of free metal ions with specific target proteins. Some of the cells like muscle, liver, renal tubular and gastrointestinal cells which transports trace elements are inclined to toxicity. Most of the elements are found in cells and accumulate in the tissues after exposure to heavy metals. Blood or urine samples just can reflect the current metal concentration of the body fluids. Hair samples can reflect past exposure but cannot give any idea about body accumulation. For these reasons, tissue studies is important in reflection of chronic metal exposure [26].

Materials and Methods

The 119 liver samples were collected in 7 months period from the autopsy cases, which exclude any kind of intoxication, received to Forensic Medicine Ankara District mainly from city of Ankara and the other suburban cities. The study sample was totally comprised of Caucasians and study design was approved by the institutional ethics committee (approval No:104–2716 in 2006). Autopsies were performed in accordance with the principles of the Declaration of Helsinki. A small questionnaire for gathering the demographic information was given to the family or relative of each autopsy cases. Phsysical conditions of cases like age, gender and body mass index (BMI) as well as personal factors like smoking habit, occupational risk and settlement were noted on a template survey document and signed by the family/relatives. Building workers, car painters, ironworkers and factory workers considered as occupationally risky group. Age and BMI values of the individuals are grouped in itself for the statistical analysis. Body mass index was calculated by dividing weight of cases to the square of their height. The BMI value between 0 and 24.9 accepted as normal and above 24.9 accepted as an overweight. The cases also grouped to their age; between 18 and 34.9 is first group, 35-59.9 is second and above 60 assessed as third group. According to settlement parameter, they are grouped as urban/suburban.

The liver tissue samples were collected during autopsy in 3cc capped polypropylene eppendorf tubes and stored at -20^{0}C until the analysis [27].

Tissue samples in eppendorf tube were thawed at room temperature before the sample preparation for AAS. Wet tissues were weighed on microbalances and transferred to the glass tables. Weighed tissues were dried in drying-oven at 75^{0}C with the glass tables for 24 hours. They were weighed again by microbalance to determine their dried weight and were taken to the teflon tubes of microwave oven. Acidic ingestion were performed in microwave oven by adding 10ml 65% HNO3 on the tubes.

Ingested liquefied samples were transferred into screw cap polypropylene tubes and completed to 25 ml by bidistilled water. All samples were stored at +4^{0}C in refrigerator before the analysis. Flame Ionization Technique were used to determine the level of copper and zinc and Graphite

Furnace Atomic Absorption Technique were used to determine the level of lead and cadmium. Argon was used as a medium gas in Graphite Furnace and SPSS15 statistical programme used for evaluation of the results.

Results

Relationship between Gender and Tissue Metal Concentrations

Relationship between gender and tissue metal concentrations is given at Table 1. According to the statistical evaluation, copper and lead levels of liver tissue were significantly higher in males, compared with females.

Relationship between Age and Tissue Metal Concentrations

Three age groups (18-34.9, 35-59.9 and 60-higher) were identified and results were given at Table 2. Liver copper levels were found to decrease with age.

Relationship between BMI and Tissue Metal Concentrations

Body Mass Index (BMI) was found by dividing weight in kilograms by height in meters squared and as BMI between 0-24.9 were classified as normal, greater values than 24.9 were accepted as overweight. There was a negative correlation between liver tissue copper levels and BMI.

Table 1. Relationship between gender and tissue metal concentrations (* p<0,05)

Metal	Gender	N	Mean Value	SD	Maximum	Minimum	P
Cu	Male	94	30,6	18,7	122,7	8,3	
(µg/g)	Female	23	22,8	7,2	35,9	5,7	**0,050***
	Total	117	29,1	17,3	122,7	5,7	
Zn	Male	94	214	76,1	627	95	
(µg/g)	Female	25	224	115,2	662,5	103	0,622
	Total	119	216	85,3	662,5	95	
Pb	Male	94	0,42	0,31	1,41	0,030	
(µg/g)	Female	24	0,26	0,18	0,64	0,033	**0,016***
	Total	118	0,39	0,30	1,41	0,030	
Cd	Male	94	4,42	2,69	11,3	0,14	
(µg/g)	Female	25	4,22	2,63	10,2	0,19	0,115
	Total	119	4,38	2,67	11,3	0,14	

Table 2. Relationship between age and tissue metal concentrations (* p<0,05)

Metals	Age	N	Mean	SD	Maximum	Minimum	P
Cu	1	41	33,3	20,9	123	9,8	
(µg/g)	2	53	26,9	11,5	63,6	5,7	**0,049***
	3	23	23,9	13,5	67,1	8,3	
	Total	117	28,6	16,1	122,6	5,7	
Zn	1	41	220	71,6	399	102	
(µg/g)	2	54	210	62,1	390	94,9	0,803
	3	24	222	139	662	103	
	Total	119	216	85,3	662	94,9	
Pb	1	41	0,36	0,27	1,23	0,03	
(µg/g)	2	54	0,43	0,32	1,36	0,03	0,419
	3	24	0,35	0,32	1,41	0,06	
	Total	119	0,39	0,30	1,41	0,03	
Cd	1	41	4,10	2,90	11,24	0,14	
(µg/g)	2	54	4,58	2,52	11,32	0,77	0,446
	3	24	4,39	2,67	9,56	0,19	
	Total	119	4,38	2,67	11,3	0,14	

Table 3. Relationship between BMI and tissue metal concentrations (* p<0, 05)

Metals	BMI	N	Mean	SD	Maximum	Minimum	P
	Normal	47	33,9	22,8	123	9,82	
Cu (µg/g)	Overweight	72	26,7	12,3	73,4	5,70	**0,026***
	Total	119	29,5	17,5	123	5,70	
	Normal	47	218	62,2	398	113,8	
Zn (µg/g)	Overweight	72	215	97,9	662	94,9	0,858
	Total	119	216	85,3	662	94,9	
	Normal	47	0,42	0,35	1,41	0,030	
Pb (µg/g)	Overweight	72	0,37	0,27	1,18	0,033	0,403
	Total	119	0,39	0,30	1,41	0,030	
	Normal	47	4,55	3,16	11,3	0,14	
Cd (µg/g)	Overweight	72	4,27	2,32	11,1	0,29	0,628
	Total	119	4,38	2,67	11,3	0,14	

Relationship between Residential Area and Tissue Metal Concentrations

Cases were divided into two groups whether they were inhabitants of urban or rural areas. Liver tissue lead concentrations were significantly higher in cases of urban areas, compared to those in rural areas.

Relationship between Occupational Exposure and Tissue Metal Concentrations

Cases were divided into two groups whether they are occupationally exposed or non-exposed group. Probably exposed group was classified as “risky group”, while the others were classified as “non-risky group”. Construction workers, welders, metal workers, painters and industrial factory workers were included in “risky group”. Liver metal concentrations didn’t show any significant difference between two groups.

Table 4. Relationship between residential area and tissue metal concentrations (* p<0, 05).

Metals	Residential Area	N	Mean	SD	Maximum	Minimum	P
Cu (µg/g)	Rural	48	27,2	12,2	73,3	8,35	
	Urban	71	31,1	20,3	123	5,70	0,229
	Total	119	29,5	17,5	123	5,70	
Zn (µg/g)	Rural	48	209	52,8	345	103	
	Urban	71	221	102	662	94,9	0,458
	Total	119	216	85,3	662	94,9	
Pb (µg/g)	Rural	48	0,30	0,23	1,16	0,030	
	Urban	71	0,46	0,32	1,41	0,033	**0,005***
	Total	119	0,39	0,30	1,41	0,030	
Cd (µg/g)	Rural	48	4,28	2,73	10,6	0,14	
	Urban	71	4,44	2,64	11,3	0,29	0,395
	Total	119	4,38	2,67	11,3	0,14	

Table 5. Relationship Between Occupational Exposure and Tissue Metal Concentrations (* p<0, 05)

Metals	Occupational Exposure	N	Mean	SD	Maximum	Minimum	P
Cu	No	84	28,9	18,04	122,6	9,1	
(µg/g)	Yes	24	32,3	15,47	73,3	8,3	0,415
	Total	108	29,7	17,48	122,6	8,3	
	No	84	210,2	79,1	662,5	94,9	
Zn (µg/g)	Yes	24	226,8	69,5	362,4	112,2	0,354
	Total	108	213,9	77,1	662,5	94,9	
	No	84	0,37	0,27	1,41	0,034	
Pb (µg/g)	Yes	24	0,4	0,35	1,16	0,030	0,595
	Total	108	0,38	0,30	1,41	0,030	
	No	84	4,21	2,51	11,24	0,19	
Cd (µg/g)	Yes	24	4,71	3,09	11,32	0,14	0,421
	Total	108	4,32	2,65	11,32	0,14	

Table 6. Relationship Between Smoking and Tissue Metal Concentrations (* p<0, 05)

Metals	Smoking	N	Mean	SD	Maximum	Minimu	P
Cu (µg/g)	Non Smoker	44	24,5	13,9	88,2	8,35	
	Smoker	68	32,3	18,9	122	5,70	**0,021***
	Total	112	29,5	17,5	123	5,70	
Zn (µg/g)	Non Smoker	44	201	93,5	662	102	
	Smoker	68	218	64,4	398	94,9	0,258
	Total	112	216	77,2	662	94,9	
Pb (µg/g)	Non Smoker	44	0,29	0,26	1,13	0,030	
	Smoker	68	0,43	0,30	1,41	0,033	**0,013***
	Total	112	0,38	0,30	1,41	0,030	
Cd (µg/g)	Non Smoker	44	3,26	2,23	9,23	0,14	
	Smoker	68	5,31	2,76	11,3	1,44	**0,030***
	Total	112	4,38	2,67	11,3	0,14	

Relationship between Smoking and Tissue Metal Concentrations

Cases were divided into two groups whether they were smokers and non-smokers.

Autopsy results show that Liver tissue copper, lead and cadmium were significantly higher in smoking group when compared with non-smoking group (Table 6).

Relationship between Metal Concentrations

Concentration of the copper, zinc, lead and cadmium metals which belong to liver tissues and their relations were analyzed statistically. There is a positive correlation in the statistics where the relation between zinc-copper; lead-copper and lead-zinc metals are evaluated and the results p<0,01 are accepted as significant.

Tablo 7. Relationship Between Metal Concentrations (* p<0, 01)

		Cu	Zn	Pb	Cd
	Pearson Korelasyon	1	0,273	0,257	-0,077
Cu	P Değeri		**0,003***	**0,005***	0,406
	Sayı	119	119	119	119
	Pearson Korelasyon	0,273	1	0,345	0,012
Zn	P Değeri	**0,003***		**0***	0,893
	Sayı	119	119	119	119
	Pearson Korelasyon	0,257	0,345	1	-0,044
Pb	P Değeri	**0,005***	**0***		0,632
	Sayı	119	119	119	119
	Pearson Korelasyon	-0,077	0,012	-0,044	1
Cd	P Değeri	0,406	0,893	0,632	
	Sayı	119	119	119	119

Discussion

In these days, technological developments not only improve the life standards but also increase environmental pollution. Thanks to the visible effects of environmental problems in different countries, environmental pollution gains importance; but, it is still not enough. In these days, heavy metals are used in the production of most of products. This situation results in the increasing level of harmful effects on living beings. Besides, inconvenient personal habits and behaviors are threatened by this and in this context, even the smoker and the health of people who are staying with user's environment are threatened by smoking. As a matter of fact, it is observed that autopsy liver tissue examples of people who are known as smokers' include statistically high and significant amount of Cadmium, Lead and Copper. Literature review also shows parallel findings [8, 28-31]. In this context, smoking addiction must be taken as a serious problem and firstly the state politics must be improved to better parent's education on this issue because they are role models.

In our research, we observed that comparison between settlement sites and metal concentration shows that the population who lives in cities has higher significant level of liver lead.

However, Bursa, one of the most important industrial cities of Turkey which has almost 3 million inhabitants, was chosen as a subject for Yılmaz's research. It is obviously seen Yılmaz's lead level findings are lower than ours [32].When we compare Yılmaz's research with our study, levels of liver lead in Yılmaz's study are lower than our study. It can be propound to these results because of Bursa had more industrial enterprises and the usage of lead-free petrol was not widespread in 1993. When the same research is compared with our cadmium concentration, our research's results are higher than Yılmaz's. This can be an important point to explain the increase in the pollution level of nature in our country.

In literature reviews, we observe that cadmium level decreases at the liver tissues which belong to +60 age's people [33-35].

In the research which was carried out by Saltzman it is determined that men accumulate more lead than women [36]. Also in our research, we found significantly more accumulation in men's bodies like Saltzman's research (p: 0,016).

In our study, concentration of Cu in men (p: 0,050) and individuals who were smoking (p: 0,021) is found significantly high. At the same time, when the age gets older (p: 0,049) and body weights increases (p: 0,029) a significant amount of decrease is observed.

In our results showed that there is no significant difference for zinc according to gender (average value for men 214 □g/gr avarege value for women 224 □g/gr.) and these results are similar with literature [26,27,30,36-41].

Most of the countries designate daily metal exposures according to their dietary and they take precautions to prevent this issue. There is not so much data about this issue for making a detailed comparison. Moreover, the accumulation of toxic metals is also related to genetic factors [42]. For this reason, it can be said that the difference between individuals for metal accumulation is not only about exposure. Therefore, researches about groups who are living in different regions and have different genetics can be beneficial for prevention to toxic metal exposure.

To conclude, in this study; liver autopsy tissue levels of metals like cadmium and lead which are important for toxicology and environmental pollution and levels of essential elements like zinc and copper which have an important role on absorption, movement, distribution and evacuation of these metals have been researched.

Datas on levels of lead, cadmium, zinc and copper in liver tissue are evaluated together with individuals' personal information statistically to understand the accumulation on liver.

The tissues in this research are chosen from people who were under living in Ankara and its round. Because of Ankara has not so much industrial enterprises, dwellers have low risk of exposure to industrial pollution. Therefore, results can give a general idea about toxic metal exposure of Turkey's population.

Dissemination of these results through government and NGOs can raise awareness about reasons of environmental and occupational metal exposure and precautions. It has been thought that this study will create a base for other studies on heavy metal tissue profile. It is aimed to perform a study with groups living in different region.

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Conflict of interest statement
The authors declare that there is no conflict of interest.

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