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Determination of the Amount of Certain Heavy Metal Ions and Some Specific Liver Enzymes and Levels of Testesterone Hormone in the Blood Sera of Heavy Asphalt Workers and Rural Community in Van, Turkey

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Abstract: In the present study, the effects of heavy metals Lead (Pb), Cadmium (Cd) on the liver enzymes Glutamate Oxalate Transaminase (GOT), Glutamic Pyruvate Transaminase (GPT) and testosterone hormone concentration heavy asphalt workers were investigated. Research material consists of blood samples taken from Van highway asphalt workers and rural community (Bardakci). The research was conducted with attention paid to personal traits. Results of lead, cadmium, enzyme activities and hormone content in the blood samples of workers and rural people are interpreted by variance analysis with uni-variety method in Spss package software. According to the results, GOT amount in highway asphalt workers was 23.27±1.09 U L-1 while it was 23.82±1.75 U L-1, GPT amount in highway asphalt workers was 23.82±1.75 U L⁻¹ while it was 20.48±1.61 U L⁻¹ in the control group. Pb amount was significant at the rate of 14.64±0.64 μg/l00 mL in asphalt workers and 5.04±0.60 μg/l00 mL and p<0.01 in the control group; Cd amount was significant at the rate of 0.16l±0.028 μg/l00 mL in the asphalt workers and 0.038±0.026 μg/l00 mL and p<0.01 in the control group with the amounts and significance as 0.283±0.032 µg/100 mL in smoking workers and 0.038±0.021 µg/100 mL and p<0.01 in non-smoking workers; the amount and significance of testosterone was found as 631.38±41.09 ng dL-1 in non-smoking asphalt workers and 388.65±29.05 and p<0.01 in smoking workers; the amount of testosterone in non-smoking asphalt workers was 657.38±36.04 and 450.05±30.63 dL⁻¹ in smoking asphalt workers with p<0.01 significance.

Key words: Toxic elements, Van, atomic Abs., liver enzymes, hormon, Turkey

INTRODUCTION

Industrialization and increased productivity in agriculture during the last century have caused levels of toxic metals (Pb, Cd) in the environment to increase. The study described the research of the effects that the environment pollution and smoking have in cadmium, lead concentrations in human blood as well as in the correlation between levels both of them. Air pollution and smoking were very important factors for the level of Cd, Pb concentrations in blood which had an inhibitory effect in liver enzymes (GOT, GPT) (Kikuchi et al., 2003). Tobacco is also great contributor on the total level of Cd, Pb concentration in blood. Usually smokers absorb a greater amount of cadmium compared to the other ways of absorption through human body. Smoking of one cigarette, containing 1-2 µg of Cd, results in the inhalation of about 01-02 µg Cd (Kikuchi et al., 2003; Friberg et al., 1986). The amount of cadmium inhaled by industrial site residents may rise

even up to 2-5 mg in cigarette addicts (Merion, 1990). Cadmium and lead are present at low concentration in the environment. However, most lead concentrations that are found in the environment are a result of human activities. Due to the application of lead in gasoline, an unnatural lead-cycle has consisted in car engines lead is burned so that lead salts (oxides, chlorines, bromines) will originate these lead salts enther the environment through the exhausts of cars. This lead-cycle caused by human production is much more extended than the naturel lead-cycle and has caused pollution to be a worldwide problem (Dowidar *et al.*, 2001).

Lead can enter the human body through uptake of air (15%), water (20%), food (65%) and cause several unwanted effects such as anaemia, a rise in blood pressure, kidney damage, distruption the biosynthesis of haemoglobin miscarriages and subtle abortions, disruption of nervous systems, brain damage, declined fertility of men through sperm damage and learning abilities of children and behavirioul and hyperactivity

(Jarup, 2003). Evidence is being gathered as to the association of exposure to inorganic Pb with the adverse outcome later in life using both animal experimental models and human epidemiology (Gulson et al., 1998; Emory et al., 1999). Cadmium is very toxic (Stayner et al., 1992; Satoh et al., 2002). It is both hepatotoxic and nephrotoxic effects in humans and rodents (Goering et al., 1994). Cadmium include testicular damage, hypertension, atherosclerosis, osteporisis, anaemia and cancer (Waalkers et al., 1999; Goering et al., 1994).

Studies and apidemiological investigations in experimental animals proves that exposure of cadmium is a causative factor for lung, prostate and cancer (Kazantzis et al., 1988; Waalkes and Rehm, 1994; Waalkes, 2000). It is prosed that cadmium caused oxidative DNA damage by various oxygen species, increasing lipid peroxidation, induction of oxidative stress via glutation consumption and inhibition of the enzymes responsible from DNA repair (Kasprzak, 1991; Bagchi et al., 1996). Heavy metals are found to affect enzymes in various ways and positive metals are reported to change enzyme activity by binding with enzymes and inhibit enzymes by destroying multi-enzim system (Spivey Fox, 1987). Cadmium especially has quite a number of negative effects on TSH.

Testosterone is a hormone synthesized by stimulation of LH hormone from leydig (interstitial) cells (Laskey *et al.*, 1984). This research was undertaken to investigate the age dependence of the liver enzyme (GOT, GPT), toxic metals (Pb, Cd), hormon (testosteron) in city and urban city (Bardakci) populations as an index of internal exposure. Toxicological investigations are obviously required in adults or old who have been exposed to significant amounts of toxins. This is important to allow comparision with the results obtained during investigations of groups following suspected exposure.

MATERIALS AND METHODS

Blood samples from 30 asphalt workers at 35-40 age range with 20 years of working experience in Van highways were used in the study and blood samples from 31 healthy people at 35-42 age range living in the rural area (Bardakci) 8 were collected as the control group. Conditions like the number of smokers (28), the number of non-smokers, age, height, weight, cigarette and alcohol consumption and health state were also taken into account in the patient group. Plasma samples from both groups were centrifuged in 1-2 h and sera are formed to determine enzyme activities (GOT, GPT) and testosterone hormone activity. Moreover, the remaining sera were kept at -20°C in order to measure the amounts of lead and cadmium.

Determination of GOT: α-keoglutarate reacts with L-aspartate to form oxaloacetate and L-glutamate then oxaloacetate reacts with NADH+H to form Malate and NAD. The kit used in the study was provided from Boehringer-Manheim Company with 1876848 serial No. 37°C was set during the test and a mixture was obtained by adding 0.2 mL serum to the buffer solution (tris buffer solution) used in the experiment. First, a reading was performed against air at 340 nm to determine enzyme activity.

Then other readings were performed at certain intervals to finish the determination process of GOT enzyme (Anonymous, 1993a).

Determination of GPT: α-oxoglutarate reacts with L-alanine to form L-glutamate and Pyruvate then Pyruvate and NADH+H react generating Lactate and NAD. The kit used in the study was provided from Boehringer-Manheim Company with 1876805 serial No. 37°C was set during the test and a mixture was obtained by adding 0.2 mL serum to the buffer solution (tris buffer solution) used in the experiment. First, a reading was performed against air at 340 nm to determine enzyme activity. Then other readings were performed at certain intervals to finish the determination process of ALT enzyme (Anonymous, 1993b).

Determination of hormone (testosterone)

Testosterone determination method: DPC brand Testosterone DC immulite 2000 kits were used (Laskey and Phelps, 1991). Test procedure were applied according to the commercial kit.

Statistical analyses of all data obtained by measurements and calculations are calculated by variance analysis with uni-variety method in Spss package software (SPSS, 1999).

Determination of lead and cadmium in serum: About 5, 10 and 20 μ g/100 mL standard lead solution was prepared for the determination of lead amount and 0.5, 1 and 1 μ g/100 mL standard cadmium solution was prepared for the determination of cadmium amount. Sera of blood samples are taken by centrifuging them twice in 10 min at 3000 rpm. The sera were put into 5 mL tubes and diluted with 1% Tritron-X100 solution. Then flame atomic absorption spectrophotometry was used for measurements (Stevens *et al.*, 1972).

RESULTS AND DISCUSSION

Lead and cadmium values in blood samples taken from Van highway asphalt workers and Van rural area residents, activities of specific liver enzymes (GOT, GPT) in blood sera and testosterone hormone activities

Table 1: Arithmetic means values (X) and standard deviations (Sx) of serum GOT, GPT and Pb, Cd and testosterone amounts

Characteristics	No	GOT (U L ⁻¹)	GPT (U L ⁻¹)	Pb (μg/100 mL)	Cd (µg/100 mL)	Testosterone (ng dL ⁻¹)
Patient	30	23.27±1.09	23.82±1.75	14.64±0.64	0.161±0.028	510.17±25.16
Smoker	20	22.15±1.26	21.65±2.02	14.08 ± 0.74	0.283 ± 0.032	388.65±29.05
Non-smoker	10	24.40±1.78	26.00±2.86	14.19±1.04	0.038 ± 0.021	631.70±41.09
Control	31	23.27±1.33	20.48±1.61	5.04±0.60	0.038 ± 0.026	553.72±23.65
Smoker	18	23.38±1.33	19.27±2.13	5.20±0.78	0.043 ± 0.034	450.05±30.63
Non-smoker	13	23.15±1.56	21.69±2.51	4.88±0.92	0.032 ± 0.040	657.38±36.04

[±] Values are expressed as X±Sx

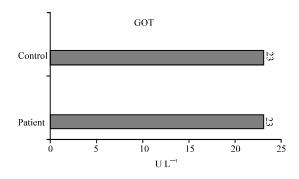


Fig. 1: Mean GOT values of patient and control groups

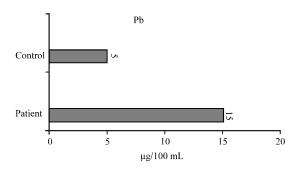


Fig. 2: Mean Pb values of patient and control groups

are shown in Table 1 and Fig. 1-5. Statistical calculations are performed by variance analysis with uni-variety method in Spss package software (SPSS, 1999). According to the Table 1, GOT amount in highway asphalt workers was $23.27\pm1.75~\rm U~L^{-1}$ while it was $23.82\pm1.75~\rm U~L^{-1}$, $20.48\pm1.61~\rm U~L^{-1}$ in the control group. According to the Table 1, Pb amount was significant at the rate of $14.64\pm0.64~\mu g/100~\rm mL$ in asphalt workers and $5.04\pm0.60~\mu g/100~\rm mL$ and p<0.01 in the control group. According to the Table 1, GPT amount in highway asphalt workers was $23.82\pm1.75~\rm U~L^{-1}$ while it was $20.48\pm1.61~\rm U~L^{-1}$, $20.48\pm1.61~\rm U~L^{-1}$ in the control.

According to the Table 1, Cd amount was significant at the rate of $0.161\pm0.028~\mu g/100~mL$ in the asphalt workers and $0.038\pm0.021~\mu g/100~mL$ and p<0.01 in the control group with the amounts and significance as $0.283\pm0.032~\mu g/100~mL$ in smoking workers and $0.038\pm0.021~\mu g/100~mL$ and p<0.01 in non-smoking workers. According to the Table 1 the

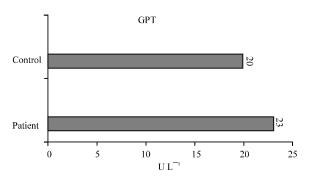


Fig. 3: Mean GPT values of patient and control groups

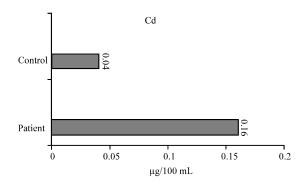


Fig. 4: Mean Cd values of patient and control groups

amount and significance of testosterone was found as 631.38±41.09 dL⁻¹ in non-smoking asphalt workers and 388.65±29.05 dL⁻¹ and p<0.01 in smoking workers; the amount of testosterone in non-smoking asphalt workers was 657.38±36.04 and 450.05±30.63 dL⁻¹ in smoking asphalt workers with p<0.01 significance. While carrying a great importance for daily life practices, metals bring excessive burdens as well. A great danger lying ahead of living beings in settlements full of environmental problems is heavy metal contamination (e.g., lead and cadmium). These metals may cause poisoning and even death if they enter the body by any means.

Poisoning is a frequent case in workers handling lead compounds in the country Guray (1996). Certain criteria should be established with regard to toxication conditions and the fact that lead is a poisonous substance taking effect by accumulating in the body.

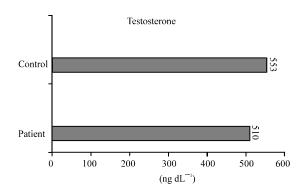


Fig. 5: Mean testosterone values of patient and control group

Lead and cadmium not only enter into the bodies by means of exhausts and similar ways they may also be taken in through foods sold in the open. Leroyer et al. (2001) found in a study conducted on 301 men and 300 women that average blood Pb amount is 74 µg L⁻¹ in males and 49 µg L⁻¹ in females, Pb amount exceeds 100 µg L⁻¹ in 30% of males and 12% of females stating the importance of alcohol and cigarette (20 and over) consumption. Truckenbrodt et al. (1984) conducted a study on 96 lead enterprise workers and 46 people living in rural area and respectively found the amount of blood lead amount as 646 and 78 µg L⁻¹ with an emphasis on a close correlation between this metal, Zn and Mn. Qu et al. (1993) found in a study in China that blood lead amount in non-smokers is 92.3 µg L⁻¹, Cd amount is 0.94 µg L⁻¹ and Pb amount of smokers is 123.4 ug L⁻¹ and Cd amount is 2.61 ug L⁻¹. Guo and Jiang (2000) concluded in a research in China that blood lead amount is higher in men compared to women and non-smokers.

According to present findings shown in Table 1, blood Pb values in the patient group (14.64 µg/100 mL) were considerably high compared to the values obtained from control group (5.04 µg/100 mL) (p<0.01) however, smoking or non-smoking conditions are not found to have significant effects on Pb amount. These results were parallel with the findings of Leroyer et al. (2001), Mortada et al. (2002), Truckenbrodt et al. (1984), Hallen et al. (1995), Chlopicka et al. (1998), Bergomi et al. (1989), Paulsen et al. (1996), Elinder et al. (1983), Khandekar et al. (1987), Guo and Jiang (2000), Maravelias et al. (1989), Jamil et al. (1987), Qu et al. (1993) and Fischer et al. (2003) in terms of environmental pollution, occupations of subjects and findings related to their environment but there is a contrast regarding the influence of smoking on lead.

The results of the present with study related to the influence of smoking on Pb show parallelism with studies conducted by Cerna et al. (2001), Shimbo et al. (2000), Higashikawa et al. (2000), Baecklund et al. (1999), Suna et al. (1991) and Marcheggiani et al. (1990). Cadmium is typically used in industrial applications, galvanizing technology, paint, lacquer, plastic compounds and nickel-cadmium batteries and also as yellow-orange pigment carriers. It is released to the atmosphere, water and to the ground with certain phosphate fertilizers during mineral were smelting and waste incineration (Sinell, 1985). Despite all the dangers it poses, cadmium widely used in industrial applications (Friberg et al., 1986). Cadmium's poisonous effects typically occur as renal and pulmonary disorders following prolonged exposures. Excessive cadmium intake may lead to hypertension, proteinuria, emphysema, osteomalacia, prostate and lung cancer cases (Wrig and Brothen, 1988). Fischer et al. (2003) performed a research within the where abouts of industrial site in Bulgaria to find the below results: blood cadmium amount in site residents and workers was $0.38 \mu g L^{-1}$ while it is $0.31 \mu g L^{-1}$ outside the industrial site and blood Pb amoun was measured as 24 and 14 µg L⁻¹, respectively. In another study emphasizing that smoking particularly leads to an increase in Cd amount Fromme et al. (1997). Cd amount was calculated as 0.25 μg L⁻¹ in non-smokers and 0.69 µg L⁻¹ in smokers. In a study conducted in Spain, Alonso et al. (2001) found 9.8 µg dL⁻¹ blood lead value in the adult residents of an area contaminated with lead and cadmium and 6.8 µg dL-1 value in the control group. Cd results of the present study show 0.161 µg/100 mL Cd amount in the highway workers and 0.038 µg/100 mL Cd amount in the control group. The difference between the two groups was significant at the rate of p<0.01, the difference between the smoking workers 0.283 µg/100 mL and non-smoking workers 0.038 µg/100 mL were also significant (p<0.01); it was found as 0.043 µg/100 mL in control group smokers and 0.032 µg/100 mL in control group non-smokers creating a significant difference (p<0.01) between smokers and non-smokers.

According to the results, environmental pollution and smoking influences particularly Cd amounts. These results were in parallel with the findings of Suna *et al.* (1991), Chlopicka *et al.* (1998), Paulsen *et al.* (1996), Benes *et al.* (2001), Cerna *et al.* (2001), Barany *et al.* (2002), Guo and Jiang (2000), Borjesson *et al.* (2001), Wittman and Hu (2002), Higashikawa *et al.* (2000) and Fischer *et al.* (2003). Kiss and Ossipento (1994) stated that toxic metal ions harm proteins and enzymes in

nucleic acids. Viarengo (1985) put forward that heavy metals affect enzymes in a different way and positive metals change enzyme activities by binding with enzymes and inhibit enzymes by destroying multi-enzyme systems. Heavy metal ions reduce the enzyme activities as oxidizing enzyme poisons (Harper, 1975). Enzymes have vital functions. Therefore, in the present study GOT GPT enzymes wereanalsed particularly. There was no significant difference between the patient and control group in GOT amounts according to Table 1.

However, though not statistically significant, GPT amounts were high in non-smokers in both groups and the patient group. This shows that smoking reduces the amount of enzymes also hints at the situation that this difference may be caused by uneven numbers of smokers and non-smokers in the related groups and the findings were in contradiction with previous researches (Kiss and Ossipento, 1994; Spivey Fox, 1987; Viarengo, 1985; Massony et al., 1995). Thus, it is concluded that cigarette and heavy metal exposure related research on evenly distributed groups shall be fruitful to clarify the subject. The study also covers the impact of cadmium on Testosterone hormone. Telisman et al. (2000) observed in a study conducted in Yugoslavia that high amounts of Cd negatively affect the number, density and motility of viable sperm cells and testosterone and prostate secretion. Significant reduction (p<0.01) in testosterone amounts were found in smokers compared to non-smokers according to Table 1. The above mentioned literature on the subject ensured the logical and completely natural evaluation of the study results.

CONCLUSION

It is of utmost importance to make further researches regarding the negative effects of heavy metals in the city and raise the awareness of public capital. This study aims to reveal the effects of heavy metals both on enzymes and the testosterone hormone. For this purpose, heavy metal accumulations of heavy highway asphalt workers are calculated. Researchers believe this study will be helpful for further researches. Hence, it is greatly important that highway workers be more careful and takes necessary measures while working. This study also reveals that people are exposed to heavy metals in a considerable extent due to environmental pollution, human actions and smoking. Moreover, it is concluded that especially Pb amount is high in asphalt workers, Cd amount is excessive in smokers and smoking negatively affects the amount of testosterone via Cd.

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