1. INTRODUCTION

Lead (Pb) is a widespread toxic metal in the human environment, getting there as a result of industrial processes (production of batteries, paints, varnishes, anti detonation agent in gasoline) (1). In terms of exposure outside profession lead is introduced into the body with food and drinking water and/or nearby roads. Absorbed lead accumulates in the body and is deposited in bones and teeth (95%), with a long biological half-life (10-20 years) and can be re-mobilized and appear in the circulation (2). Especially vulnerable populations are children, where adverse effects may occur at lower concentrations of lead in blood than in the adult population.

Toxic effects of lead and effect on different biological systems and functions are well known (3). The current findings suggest that lead, even at low concentrations in the blood, exerts neurotoxic effects, activates protein C kinase (4), reduces mental and physical functions and/or have adverse effect on the immune system (5). It is known that lead has vasoconstrictive effect on blood vessels, which can affect the increase in blood pressure, or result in a nephrotoxic effect (6,7). Other biological effects of inorganic lead include genetic toxic and carcinogenic effects (8), a mechanism that is not sufficiently known, and possibly involves indirect damage to DNA, changing the stability of chromosomes (9) or DNA repair processes (10). The International Agency for Research on Cancer-IARC in 2004 based on limited data on the carcinogenicity of lead in humans and sufficient data on laboratory animals conducted by amending the earlier classification of inorganic lead, putting it in group 2A, as probably carcinogenic to humans, instead of the earlier classifications-possible carcinogen to humans (2B). Organic lead (molecules containing carbon-hydrogen bond) remained unclassified, with inadequate data on the carcinogenicity to humans and animals (11). Also described are effects of lead on reproduction, including spontaneous abortion in women and the impact on male reproductive health, with a change of semen quality in the moderate exposure to lead, and acting on the axis hypothalamus-pituitary-gonads (12). The hematological effects of lead in blood vary from subclinical effects to manifested changes, including inhibition of dehydratase enzyme δ-aminolevulinic acid (ALAD), elevation of erythrocyte protoporphyrin levels (EP), an increase of urinary δ-aminolevulinic acid (ALA), reducing the production of hemoglobin and anemia (6). Determined LOAEL-lowest observed adverse effect level concentrations of lead in blood, based on recommendations from the American FDA Food and Drug Administration for adults is 300 mg/L-1, while for children recommended value is 100 mg/L-1 (13).
The aim of this study is to assess exposure to lead among occupationally exposed persons based on biological indicators of exposure (BPb) and indicators of lead effects (ALA), as well as the possible impact of lead on standard hematological parameters.

The study was conducted in accordance with bioethical principles of good clinical practice.

2. Sample and Methods

Conducted is examination of workers (n=73, n=81), male employees at the petrol stations in Sarajevo, at the workplace of fuel pourer. Periodic examinations were performed in 2003 and 2008, on the same population of subjects, from the same job, same location and with the same content inspection. Characteristics of respondents (n=73) (n=81) were (mean ± SD): age 35.1±11.4 years (range 23-62 years), total work experience 22.0±7.4 years (range 1-31 years), duration of exposure at the workplace 12.7±5.6 years (1-27 years). Among the respondents 70% of them were active smokers, and only 30% of nonsmokers.

For both groups of patients were determined the concentration of lead in air, which had a value of <0.01 mg/m³ at all points of measurement (OEL—occupational exposure limit of 0.01 mg/m³). From the study subjects were taken blood samples for analysis of blood lead concentration (BPb), and ALA in urine analysis, analytic processing is performed at the Institute of Occupational Health of Sarajevo Canton, Laboratory for Toxicology and hygiene of working environment. BPb analysis was carried out using electro thermal atomic absorption spectrometry with Zeeman correction of nonspecific absorption (ET-AAS) (14). The value of ALA in urine was determined by a standardized method (15).

In accordance with the aims of research, the subjects underwent hematological analysis, determination of activity of γ-glutamyl transferase (GGT) and blood pressure.

Hematological parameters include determining the number of erythrocytes in blood (E), hemoglobin (Hgb), hematocrit (Hct), leukocyte count (L), lymphocyte count (Ly), as well as determining the mean corpuscular volume (MCV), mean hemoglobin in erythrocytes (MCH) and high concentrations of hemoglobin in red erythrocytes (MCHC). Determined is the activity of γ-glutamyl transferase (GGT), which is observed as an indicator of the effect of alcohol consumption on liver parenchyma. In particular the data are collected about the duration of smoking.

Assessment of statistical significance of mean values was performed using the Student t-test. Using Pearson correlation coefficient we tested the association of the measured parameters. Statistical analysis was performed using the program Sigma Stat 3.5.

3. Results

Demographic characteristics of respondents, the overall and exposure work experience at the current workplace, and smoking experience, alcohol consumption and blood pressure are shown in Table 1. Basic characteristics of respondents in terms of employment and exposure period, the duration of smoking and alcohol consumption and blood pressure were not significantly different, except the duration of smoking.

Biological markers of exposure and effect of lead (BPb, ALA), and observed hematological parameters are shown in Table 2. Observed is a statistically significant difference between the biological markers of exposure and effect (BPb, ALA) in the observed group of subjects (p<0.041). The same trend of statistical significance refers to the observed hematological parameters (Hgb, Hct, MCV, MCHC, L), including GGT.

Correlation relationship of biological markers in all subjects is shown in Table 3. There was a positive correlation between BPb and age (r=0.33, p<0.05), work experience (r=0.31, p<0.05) and years of exposure (r=0.40, p<0.05). Observed is a positive correlation between BPb variables and GGT.
(r=0.0301, P<0.05). Correlation of BPb is not determined toward duration of smoking, alcohol and blood pressure. In case of standard hematological parameters were found positive correlation between BPb and the number of erythrocytes (r=0.0241, P<0.05), hemoglobin (r=0.0201, P<0.05) and MCH (r=0.0213, P<0.05). Correlation is not determined by the number of leukocytes, lymphocytes and blood pressure. Correlation relationship between the observed parameters of ALA has not been established.

4. DISCUSSION

Determined mean BPb of 154 patients was 50.5 μg/L-1, with no statistically significant differences between BPb in two groups (40.4, 59.6, P<0.05) (Table 2).

Measured mean concentrations of BPb is 7.9 times smaller than the average value for the male population occupationally exposed to lead and less than twice the reference value for the general population. At the same time, the increase in the mean BPb in humans in relation to the population of industrialized countries such as Sweden (25 μg/L-1), USA (27 μg/L-1), Germany (36 μg/L-1), Italy (45 μg/L-1), Poland (27 μg/L-1) (16). Biological threshold BPb for the occupationally exposed population is 400 mg/L-1 for men and 300 μg/L-1 for women of reproductive age. Established values of BPb in our study may be associated with a greater presence of older cars in traffic, increasing the use of leaded gasoline (0.6 g/L of lead) and lead entering the body by secondary, not occupation related, routes and especially smoking and alcohol use. The obtained data show that there is no correlation between BPb and smoking duration, and alcohol consumption, although it is known that alcohol changes the distribution of lead in the body and increases the urinary excretion of lead as a result of the transient increase of redox-potential of the body (17). Other reasons that may have a share in the BPb level are the different places of residence or with different exposures in the workplace. In our study there was no difference in the concentration of lead in air at the location of the job.

Hematologic changes can be considered as most famous biological effects of lead on health. Anemia found in lead exposed persons is of normochrom, normocyt and microcyte type. Reduction of hemo synthesis occurs at the concentration of lead in blood of 500μg/L. Pathophysiological mechanisms of action of lead points to the disposal of lead in the circulation at the surface of erythrocytes in the form of aggregates of lead sulphate and the binding biology and phosphate ligands in the erythrocyte membrane, which increases the fragility of erythrocytes and erythroblasts (18). Our data show a positive correlation between hemo-globin concentration and BPb (r=0.0201, P<0.05). The correlation was not found by the value of hematocrit and leukocyte count, where there are reports of immune response with prolonged exposure to lead (19). There is a positive correlation between BPb and age, work experience (r=0.330, 0.319, P<0.05) and years of exposure, which is correspondent with previous research (20). A significant correlation between BPb and GGT can be interpreted as a possible indicator of hepatotoxic effect of alchol consumption. Biological effects of lead are manifested also with increasing blood pressure and the occurrence of hypertension in the exposed persons and the general working population, the BPb concentrations greater than 370 μg/L-1 and BPb concentrations of 100 μg/L-1 results increase in systolic pressure by 5 mmHg (21). Our results show no correlation between BPb and blood pressure.

5. CONCLUSION

Research results indicate increased levels of lead in blood of workers at petrol stations, which are below the biological limit values for occupational population, but were higher compared to the average values of BPb workers in industrially developed countries. The reasons for the substantial intake of lead due to the presence of a large number of older cars in traffic, greater use of leaded gasoline, which increases the concentration of lead in the air, but also other possible ways of input.

REFERENCES