DOI: 10.2478/10004-1254-59-2008-1866

Scientific Paper

ADVERSE EFFECTS IN WORKERS EXPOSED TO INORGANIC LEAD

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Received in December 2007
Accepted in February 2008

This paper describes a retrospective cohort study comparing 60 workers occupationally exposed to inorganic lead and 60 matched controls. All subjects were assessed using data obtained from a specially designed Questionnaire for lead exposure and toxic effects assessment, physical examination, spirometry, ECG, and laboratory tests including blood lead level (BLL) and biomarkers of lead toxic effects. Muscle pain, droopiness, and work-related nasal symptoms were significantly more frequent in lead workers. The prevalence of lung symptoms was higher in lead workers than in controls, but not significantly (20 % vs. 6.6 %, respectively). Mean values of BLL and δ-aminolevulinic acid (ALA) were significantly higher in lead workers. The activity of δ-aminolevulinic acid dehydratase (ALAD) in lead workers was significantly lower than in controls. Abnormal of BLL, ALAD, and ALA were more frequent in lead workers, with statistical difference for BLL and ALAD. Inverse correlation was found between BLL and ALAD, and positive correlation between BLL and age, years of employment, and years of exposure. Inverse correlation was found between ALAD and age, years of employment, years of exposure, blood pressure, alcohol consumption, and years of alcohol consumption. Changes in spirometry correlated inversely with BLL. A positive correlation was found between BLL and erythrocyte count and haemoglobin concentration, whereas it was inverse for ALAD and haemoglobin concentration. A significant difference was found for BLL and ALAD, with a very high odds ratio (14.64 and 7.23, respectively) and high relative risk (4.18 and 3.08, respectively). Our data have confirmed the association between occupational lead exposure and deviation in specific biological markers of lead effect and between the role of occupational exposure in the development of adverse effects.

KEY WORDS: *ALA, ALAD, biological markers, blood lead, occupational exposure, toxicity, δ-aminolevulinic acid dehydratase*

Lead is a natural constituent of the earth's crust, but once mined and transformed into man-made products, lead becomes highly toxic (1). Lead dispersed through gasoline exhausts, smelter emissions, and paint removers never fully disappears from our environment nor has the man evolved a good biological system to offer any protection from it (2). The contact with lead and its compounds within different conditions and circumstances can result in acute, sub-acute, and chronic occupational lead poisoning, present in the process of lead production and use or in non-occupational lead poisoning, present in everyday life through emissions by lead smelting plants, exhausts by motors running on ethyl fuel, and through soil via food or drinking water (3). Since lead has been phased out as a gasoline additive (tetraethyl lead) since the 1970s and its use in paint and food containers has been curtailed, blood lead concentrations have decreased significantly in general population, but other sources of lead and its unknown toxicity threshold continue to make lead an issue of public health. (4). The beginning of production in the

lead smelting plant "MHK Zletovo" in the municipality of Veles in 1972 gave rise to issues related to occupational exposure to inorganic lead. Occupational lead exposure mostly occurs in lead smelting plants and battery factories, as well as in renovation of old houses, when workers inhale or ingest fumes and dust contaminated with lead. Occupational risk exists where lead and its compounds are present in the form of vapour or aerosol (lead fume, lead dust) in the working environment, especially in lead ore smelting (5, 6). The only larger complex in Macedonia which is an important source of lead pollution is the lead and zinc smelting plant in Veles with estimated lead emission of 83 tonnes per year according to the 1996 National Environmental Action Plan (NEAP) (3). The average lead emission in Macedonia in 2003 was 16 g per resident (3). Occupational lead poisoning is almost without exception chronic and occurs after exposure of several weeks or months (7). Lead is one of the best-studied toxic substances (8), with very low levels of detection (9), and is a confirmed multi-target toxicant with effects in the gastrointestinal, haematopoietic, cardiovascular, nervous, immune, and reproductive system and the kidneys (10-13). Blood lead level (BLL) is most frequently used bioindicator of recent lead exposure (14), the most appropriate indicator of actual (present) or previous permanent lead exposure, but also a good indicator of lead body burden. Biological markers of lead effects are the activity of δ-aminolevulinic acid dehydratase (ALAD), free erythrocyte protoporphyrin (FEP), pirimidin-5'-nucleotidase in erythrocytes, and the concentration of aminolevulinic acid (ALA) and coproporphyrin in urine (15). At an average or high lead exposure FEP and zinc protoporphyrine (ZPP) concentrations increase, but are not sufficiently sensitive or specific to be primary indicators of lead effects (16, 17). The lowest observed adverse effect level (LOAEL) is a health-based level of concern (18). According to the US Food and Drug Administration (US FDA) the LOAEL for blood lead concentration is 100 μg L^{-1} in children and foetuses and 300 μg L^{-1} in adults (19). Lead inhibits three enzymes in the haem biosynthesis (ALAD, coporphyrinogen oxidase, and ferrochelatase), but its effects on ALAD are most profound (20). As erythrocyte ALAD activity is inhibited, ALA levels in blood increase, leading also to elevated levels of ALA in the urine. The threshold for detecting elevated blood and urinary ALA levels is 400 μg L^{-1}, but may be as low as 150 μg L^{-1} to 200 μg L^{-1}. Increased coproporphyrin levels are elevated in individuals with blood lead concentrations of 400 μg L^{-1} (21). ALAD is one of the most sensitive indicators of lead effect with the threshold BLL of less than 100 μg L^{-1}. In her study of occupational lead exposure in the smelting plant in Veles in 1997, Isjanovska found a statistically significant difference between average ALAD blood values in the exposed and unexposed workers ($p<0.001$) (6). Results of a 2004 study on lead exposure conducted by the Institute of Occupational Health of Skopje showed a higher frequency of abnormal ALAD in lead workers from a smelting plant (58.5 %), than in urban adult population without occupational exposure (14 %; $p<0.001$) (22). Occupational exposure to inorganic lead is still a burning issue in metal production and certain aspects of lead toxicity have still not been clarified. The aim of this study was to identify the association between occupational exposure to lead and abnormalities in specific biomarkers of lead effect, to determine the role of occupational exposure in the development of lead-related adverse effects development, haematopoesis in particular, and to establish factors that may modifying the expression of lead toxicity.

SUBJECTS AND METHODS

Study design

A retrospective cohort study was carried out in two groups differing in risk and exposure to lead at the Institute of Occupational Health, Skopje - WHO Collaborating Center from November 2004 to December 2006. All subjects were assessed using a specially designed Questionnaire for the assessment of lead exposure and toxic effects, aimed at early detection of health damage caused by lead. All study subjects underwent physical examination and laboratory and toxicology tests. The influence of sex on the study results was not evaluated.

Questionnaire for lead exposure and toxic effects assessment

For the research purposes we have used a specially designed questionnaire that was filled in by a medical doctor, and contained data about demographics, accompanying diseases, work history (occupation, workplace, duration of exposure, and total duration of employment, previous workplace, if any, and duration, hazards at the workplace), smoking status (active smoker, ex-smoker, non-smoker, number of cigarettes

per day, duration of smoking), alcohol consumption (quantity and frequency), risk information, work organization, absenteeism, medical and family history, work-relatedness of the symptoms, and use of preventive measures. The questionnaire was useful for obtaining data about specific occupational risks and health status in the exposed workers, association between lead exposure and abnormalities in specific lead biomarkers, lead toxic effects and possible modifying factors for their expression. This questionnaire was carried out in the municipality of Veles within a comprehensive preventive programme of occupational and environmental toxicology of heavy metals, supported by the Ministry of Health of the Republic of Macedonia.

Subjects

The exposed group (Group I) consisted of 60 lead workers of the smelting plant of Veles, who worked in lead production and refining (occupationally exposed to inorganic lead); 51 were men and nine women, with the following averages: age=(45.1±7.6) years, total employment duration=(22.8±9.2) years, exposure duration or years on the current workplace=(19.2±7.8) years.

The control group (Group II) consisted of 60 workers employed in different services and industries in Veles, other than lead industry, without any occupational exposure to lead (living in urban areas where leaded petroleum can still be found and having lead-emitting industrial facilities in the local environment); 50 were men and 10 women, aged (42.2±8.7) years, with total employment duration of (18.9±9.7) years.

The two groups did not significantly differ in demographic characteristics, environmental exposure, total duration of employment, smoking habit, and alcohol consumption. The study included only those subjects who had complete data from all examinations and tests. All 120 qualifying subjects volunteered for the research and gave their oral consent. The study was approved by the bioethical committee and performed according to the Declaration of Helsinki. The basic criteria for determining occupational risk factors in the study were data collected by the questionnaire for lead exposure and toxic effect assessment.

Clinical examinations

Physical examination of each subjects included the eyes (redness, pruritus, tearing, discharge, and photophobia), nose (sneezing, nasal itching, rhinorrhoea, and nasal blockage), lungs (wheezing, breathlessness, chest tightness, coughing and phlegm), and skin (rash, pruritus and inflammation). It was followed by spirometry using the Ganshorn SanoScope LF8 (Ganshorn Medizin Electronic GmbH, Germany) which included measurement of the forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), FEV_1/FVC ratio, maximal expiratory flow at 50 %, 25 %, and 25 % to 75 % of FVC (MEF_{50}, MEF_{25}, and MEF_{25-75}, respectively), in all subjects. The results are expressed as percentages of the predicted values set by the European Community for Coal and Steel (ECCS) norms (23). Finally, all subjects underwent ECG testing at steady state.

Laboratory testing

Blood lead level was determined in the Republic Institute for Health Protection, Skopje using a PERKIN ELMER 4100 HGA 700 atomic absorption spectrometer (AAS) with an auto-sampler AS-70. Venous blood (about 2 mL) was taken into a sterile vacutainer with K_2EDTA 1.5 mg mL^{-1} of blood and transported in a portable refrigerator at +4 °C on the same day. Lead was extracted into a mixture of HNO_3 and HCl under pressure using a microwave furnace PAAR PHYSICA-PERKIN ELMER designed for laboratory use (24, 25). The sensitivity of the method is $4.4x10^{-10}$ g Pb (26, 27). Biological material (venous blood and urine spot samples) was taken to determine the biomarkers of lead effect at the Institute of Occupational Health, Skopje.

ALAD activity was determined in 0.2 mL venous blood samples with heparin using the spectrophotometric method within 24 hours of sampling because of ALAD's instability. An ALA substrate was added to the haemolised blood to facilitate a reaction between ALAD and its substrate ALA, which resulted in the forming of porphobilinogen. The amount of porphobilinogen, determined by spectrophotometry at 555 nm after adding *p*-dimethyl amino benzaldehid, showed the ALAD activity (28, 29). ALA concentration in the urine was determined by condensing 1 mL spot urine samples with acetyl acetone into a pyrole compound which together with para-dimethy-aminobenzaldehyd gave a red coloured complex that was determined by spectrophotometric method at 553 nm (30). Coproporphyrin concentration in urine spot samples was determined by spectrophotometric absorption

measurement at 401 nm of the urine extract by ether and afterwards by HCl (29). Reticulocyte count was determined in blood sample (31), and count of erythrocytes with basophilic stipplings (EBS) was carried out using two mixtures: 2 g boric acid + 1 g methylen blue and 0.28 g NaOH in 100 mL of distilled water. This mixture was used to paint the blood sample on the microscopic glass and then fix it and analyse by microscope (32). Blood haemoglobin concentration was determined using the colourimetric method (33), while erythrocytes and leukocytes were counted using the haematological counter (34).

Statistical analysis

Data were analysed with descriptive and inferential statistical methods using STATISTICA for Windows release 7 and Epi Info 6. A data base was created using specific computer applications for this purpose (Delphi 5, MS Excel). Descriptive statistical analysis included tables and figures containing statistical series according to the defined variables. Continuous variables were expressed as mean values with standard deviation (SD) and nominal variables as numbers and percentages. The chi-square test (or Fisher's exact test where appropriate) was used for testing differences in frequency. A P-value below 0.05 was considered statistically significant. Risk was assessed using the odds ratio (OR) with 95 % confidence interval (CI).

RESULTS

Table 1 shows the subject demographics. The prevalence of non-work-related symptoms in the previous 12 months was not significantly higher in lead workers than in controls, except for the muscle pain and droopiness (Table 2).

Table 3 shows the prevalence of work-related symptoms in both groups over the last 12 months. Work-related worsening of the symptoms of the

Table 1 *Characteristics of the study subjects*

Variable	Lead workers (n=60)	Controls (n=60)
Sex / men/women ratio	5.6	5.0
Age / years	45.1±7.6	42.2±8.7
BMI / kg m^{-2}	25.3±3.4	26.4±3.6
Years of employment	23.1±7.2	18.9±9.7
Years of exposure	18.8±7.5	/
Daily smokers	25 (41.6 %)	26 (43.3 %)
Years of smoking	Range=0 to 40; median=8.5	Range=0 to 42; median=10.5
Cigarettes per day	Range=0 to 35; median=7.3	Range=0 to 40; median=8.2
Ex-smokers	7 (11.6 %)	9 (15.0 %)
Passive smokers	8 (13.3 %)	7 (11.7 %)

Numerical data are expressed as means with standard deviations; the frequencies of active smoking, ex-smoking, and passive smoking are expressed as absolute numbers and percentages of subjects.
BMI: body mass index.

Table 2 *History of non-work-related symptoms in 60 lead workers and 60 control subjects in the last 12 months*

Symptoms	Lead workers (n=60)	Controls (n=60)	P-value *
Headache	17 (26.6 %)	13 (21.6 %)	0.513
Insomnia	7 (11.6 %)	5 (8.3 %)	0.582
Low appetite	8 (13.3 %)	4 (6.6 %)	0.271
Muscle pain	20 (33.3 %)	8 (13.3 %)	0.041
Decreased body weight	2 (3.3 %)	0	0.161
Droopiness	22 (36.6 %)	7 (11.6 %)	0.012
Gastrointestinal symptoms	15 (25 %)	9 (15 %)	0.263
Grumpiness	8 (13.3 %)	2 (3.3 %)	0.068

Data are expressed as number and percentage of subjects with certain variable.
** Tested by the chi-square test.*

eyes, nose, lungs, and skin) was reported by 18.3 %, 28.3 %, 20 %, and 10 % of lead workers, respectively and by 6.6 %, 10 %, 6.6 %, and 6.6 % controls, respectively. A significant difference between lead workers and controls was only found for work-related nasal symptoms ($P=0.035$). The prevalence of lung symptoms was higher in lead workers than in controls, but the difference was not significant (20 % vs. 6.6 %).

Table 4 shows the reference values for biological markers of lead exposure and effect. Table 5 shows the mean values for biological markers of exposure and effect. BLL and ALA were significantly higher, and ALAD activity significantly lower in lead workers than in controls. Lead workers also had a higher rate of abnormal BLL, ALAD, and ALA, but the difference was significant only for BLL and ALAD (Table 6). The percentage of abnormal coproporphyrine, haemoglobin, reticulocytes, EBS, and erythrocyte count was equal or lower than in controls (Table 6). This is due to the fact that BLL mainly reflects current lead exposure, ALAD better reflects long-

Table 3 *Prevalence of work-related symptoms in 60 lead workers and 60 control subjects within the last 12 months*

Symptoms of	Lead workers (n=60)	Controls (n=60)	P-value *
Eyes	11 (18.3 %)	4 (6.6 %)	0.088
Nose	17 (28.3 %)	6 (10 %)	0.035
Lungs	12 (20.0 %)	4 (6.6 %)	0.059
Skin	6 (10.0 %)	4 (6.6 %)	0.543

Data are expressed as number and percentage of subjects with certain variable.
** Tested by the chi-square test.*

Table 4 *Biological markers and reference values*

Biological markers	Reference values
BLL	$<100\ \mu g\ L^{-1}$
Erythrocytes count	$(3.8x10^{12}$ to $5.8x10^{12})\ L^{-1}$
Hemoglobin concentration	(110 to 180) $g\ L^{-1}$
Reticulocytes count	(5 to 25) ‰
ALAD	(1336 to 2000) ncat
EBS count	0 to 100
ALA	$76\ \mu mol\ L^{-1}$
Coproporphyrine	$0.18\ \mu mol\ L^{-1}$

Data are expressed as certain variable with referent values.

Table 5 *Findings of biological markers in 60 lead workers and 60 control subjects*

Biomarkers	Lead workers (n=60) Mean±SD (range)	Controls (n=60) Mean±SD (range)	P-value *
BLL / $\mu g\ L^{-1}$	164±85 (33-467)	70±54 (0-277)	0.000
Erythrocytes / $x10^{12}\ L^{-1}$	4.9±0.5 (3.2-6.2)	4.8±0.4 (3.7-5.7)	1.000
Hemoglobin /$g\ L^{-1}$	149±10.6 (119-166)	149.6±12.1 (118-178)	0.948
Reticulocytes / ‰	15.1±3.5 (8-25)	14.9±5.7 (9-50)	0.848
ALAD / ncat	1238.1±647.4 (82-3990)	2096.5±910.8 (233-4036)	0.000
EBS count	30.8±54.5 (0-350)	26.8±37.2 (0-150)	0.639
ALA / $\mu mol\ L^{-1}$	45.1±32.2 (13.7-221.5)	28.1±17.8 (2.3-86.9)	0.0005
Coproporphyrine / $\mu mol\ L^{-1}$	0.06±0.04 (0.01-0.3)	0.06±0.04 (0.006-0.24)	0.681

**Compared by independent-samples t-test.*

term, cumulative lead exposure level, whereas ALA, coproporphyrine, and EBS are not specific enough, and erythrocyte count, reticulocyte count, and haemoglobin concentration are not specific at all for lead exposure. Correlations between the examined parameters and BLL, ALAD and ALA in lead workers are shown in Table 7. Inverse correlation was found between BLL and ALAD (r=-0.546, $P<0.01$), and positive correlation BLL between and age (r=0.331, $P<0.01$), years of employment (r=0.418, $P<0.01$), and years of exposure (r=0.419, $P<0.01$). Inverse correlation was found between ALAD and age (r=-0.256, $P<0.05$), years of employment (r=-0.371, $P<0.01$), and years of exposure (r=-0.343, $P<0.01$). ALAD also inversely correlated with systolic blood pressure (r=-0.262, $P<0.05$), alcohol consumption (r=-0.277, $P<0.05$), and years of alcohol consumption (r=-0.301, $P<0.05$). Body mass index, smoking, ECG abnormality, number of cigarettes per day and years of smoking did not correlate with either BLL or ALAD. Changes in spirometric parameters correlated inversely with BLL (r=-0.255, $P<0.05$). As far as standard

Table 6 *Prevalence of deviation in biological markers in 60 lead workers and 60 control subjects*

Biomarkers	Lead workers (n=60)	Controls (n=60)	P-value *
BLL	46 (76.7 %)	11 (18.3 %)	0.0001
Erythrocytes	1 (1.7 %)	1 (1.7 %)	1.00
Haemoglobin	0	1 (0.02 %)	0.319
Reticulocytes	8 (13.3 %)	8 (13.3 %)	1.00
ALAD	40 (66.7 %)	13 (21.7 %)	0.0018
EBS	6 (10.0 %)	7 (11.7 %)	0.792
ALA	5 (8.3 %)	1 (1.7 %)	0.110
Coproporphyrine	1 (1.7 %)	2 (3.3 %)	0.568

Data are expressed as number and percentage of subjects with certain variable.
** Tested by chi-square test.*

Table 7 *Spearman's rank correlation coefficients for relationship between the examined parameters and blood lead level (BLL), δ-aminolevulinic acid dehydratase (ALAD), δ-aminolevulinic acid (ALA)*

Parameters	BLL	ALAD	ALA
BLL	1	-0.546**	-0.005
ALAD	-0.546**	1	-0.071
ALA	-0.005	-0.071	1
Age	0.331**	-0.256*	-0.022
Years of employment	0.418**	-0.371**	0.026
Years of exposure	0.419**	-0.343**	0.115
Body mass index	-0,078	-0,064	0,038
Blood pressure	0.129	-0.262*	-0.029
Smoking habit	-0.002	-0.065	0.052
Years of smoking	0.096	-0.158	-0.130
Cigarettes per day	0.034	-0.066	0.027
Alcohol consumption	0.179	-0.277*	0.082
Years of alcohol consumption	0.177	-0.301*	0.107
Spirometric changes	-0.255*	0.078	-0.018
ECG abnormalities	0.106	-0.179	-0.012
Erythrocyte count	0.258*	-0.137	-0.151
Haemoglobin	0.261*	-0.322*	0.072
Haematocrit	0,157	0.209	-0.302
Leukocyte count	0.017	-0.118	-0.111

*Level of statistical significance: *P<0.05; **P<0.01*

haematological findings are concerned, a positive correlation was found between BLL and erythrocyte count (r=0.258, $P<0.05$) and BLL and haemoglobin (r=0.261, $P<0.05$), and inverse correlation was found between ALAD and haemoglobin (r=-0.322, $P<0.05$). No correlation was found between BLL or ALAD and leukocyte count and haematocrit, or between BLL and systolic blood pressure. Biomarker risks were evaluated in lead workers and controls using the odds ratio (OR) with 95 % confidence interval (CI). A significant difference was found for BLL and ALAD, with very high odds ratios (14.64 and 7.23 respectively), as well as high relative risk (RR) (4.18 and 3.08 respectively) (Table 8).

DISCUSSION

The average blood lead in lead workers (164 $\mu g\ L^{-1}$) was significantly ($P=0.000$) higher than in control subjects (70 $\mu g\ L^{-1}$) (Table 5). General population in Macedonia seems to have a higher blood lead level than in other industrially developed countries such as Sweden (25 $\mu g\ L^{-1}$) (35), USA (27 $\mu g\ L^{-1}$), Thailand (32 $\mu g\ L^{-1}$) (36), Poland (27 $\mu g\ L^{-1}$) (37), Germany (36 $\mu g\ L^{-1}$) (38) or Italy (45 $\mu g\ L^{-1}$) (39), perhaps because leaded gasoline is still widely used in Macedonia. Values of BLL and ALAD also depend on genotype variants (4). Of eight ALAD gene variants, we focused on one polymorphism that yields two alleles, designated ALAD-1 and ALAD-2, which exhibit a co-dominant pattern of inheritance (40). Ziemsen et al. (41) found that lead-exposed workers with the ALAD 1-2 genotype had higher blood lead levels than ALAD 1-1 homozygotes (440 $\mu g\ L^{-1}$ vs. 380 $\mu g\ L^{-1}$) and that ALAD 2-2 homozygotes had still higher blood lead levels (560 $\mu g\ L^{-1}$). Hypotheses based on the charge of the ALAD-2 isozyme (42) imply that the ALAD 1-2 and 2-2 genotypes are the "at-risk genotypes" at high exposure levels. The average value of ALAD (1238.1 ncat) in lead workers was significantly ($P=0.000$) lower than in controls (2096.5 ncat) (Table 5). ALAD has a highly significant correlation with BLL at very low BLL, and is also sensitive to high lead (43). The comparative advantage of ALAD with respect to other biological markers of effect seems to be its relatively higher specificity for increased lead absorption. ALAD may be inhibited by alcohol and smoking (44). However, Telišman et al. (45) found that transient inhibition of ALAD by alcohol is owed to ethanol per se, but mainly to an ethanol-induced increase in the biologically active fraction of lead accumulated in the organism. Namely, alcohol can affect the lead distribution in the body and increase urinary excretion of lead because of a transient increase in tissue redox potential resulting from ethanol metabolism (45, 46). Our data showed no correlation between ALAD and smoking habit, years of smoking, and the number of cigarettes per day, but there was an inverse correlation between ALAD and alcohol consumption (r=-0.277, $P<0.05$) and ALAD and years of alcohol consumption (r=-0.301, $P<0.05$). A strong positive correlation was found between BLL and years of employment (r=0.418, $P< 0.01$) as well as between BLL and years of exposure (r=0.419, $P<0.01$), while the correlation between these variables and ALAD was inverse (r=-0.371, $P<0.01$ and r=-0.343, $P<0.01$, respectively). As the main parameter of airway obstruction, FEV_1 is used to determine the degree of lung function impairment (47). Our results showed an

Table 8 *Biomarker risk evaluation using the odds ratio (OR) with 95 % confidence interval (CI) in lead workers and controls*

Biomarkers	Odds ratio	Relative risk (CI=95 %)	P-value *
BLL	14.64 (5.57<OR<39.60)	4.18 (2.41<RR<7.26)	0.0000
Erythrocytes	1.0 (0.0<OR<37.61)	1.0 (0.06<RR<15.62)	1.000
Haemoglobin	0.0 (0.0<OR<17.54)	/	0.315
Reticulocytes	1.0 (0.31<OR<3.21)	1.0 (0.4<RR<2.49)	0.558
ALAD	7.23 (2.98<OR<17.87)	3.08 (1.84<RR<5.14)	0.0000007
EBS	0.84 (0.23<OR<3.03)	0.86 (031<RR<2.40)	0.768
ALA	5.36 (0.58<OR<125.23)**	5.0 (0.60<RR<41.53)**	0.0938
Coproporphyrine	0.49 (0.02<OR<7.19)	0.50 (0.05<RR<5.37)	0.558

* *Tested by the chi-square test*
** *Cornfield not accurate*

inverse correlation between FEV_1 and BLL (r=-0.255, $P<0.05$), but we have not taken into account of the effect of smoking on FEV_1 and FEV_1/BLL ratio. Some studies confirmed the effect of exposure duration on the prevalence of respiratory symptoms (48). Anaemia observed in lead-exposed individuals is of the hypochromic and normocytic (also microcytic) type, and is accompanied by reticulocytosis with basophilic stippling. Reduced haem synthesis is seen at blood lead levels of 500 μg L^{-1} (49). Our data showed a positive correlation between haemoglobin concentration and BLL (r=0.261, $P<0.05$), and inverse correlation between haemoglobin and ALAD (r=-0.322, $P<0.05$), which is in agreement with the findings of Kelada et al. (4). No such correlation was seen for haematocrit and leukocyte count in exposed workers, although some authors reported immune response in long-term lead exposure (50). A positive correlation was found between BLL and age (r=0.331, $P<0.01$), and inverse between ALAD and age (r=-0.256, $P<0.05$), which corresponds to some earlier studies (35, 39). Body mass index showed no correlation with biological markers of lead exposure and effect. Lead exposure is also weakly associated with increased blood pressure and hypertension in general and occupationally exposed population. Pocock et al. (51) concluded that increased hypertension [systolic pressure greater than 160 mm Hg (21.33 kPa), diastolic pressure greater than 100 mm Hg (13.33 kPa)] was associated with blood lead concentrations greater than 370 μg L^{-1}. Morris et al. (52) reported that BLL of 100 μg L^{-1} produced a systolic increase of 5 mm Hg (0.66 kPa) in men. Our findings showed an inverse correlation between systolic blood pressure and ALAD (r=-0.262, $P<0.05$), and no correlation with BLL. A significant difference was found for BLL and ALAD between lead workers and controls, with very high odds ratios (14.64 and 7.23, respectively) and high relative risk (4.18 and 3.08, respectively). By identifying the proteins prone to bind lead, we may be able to understand lead toxicity better, find markers of lead exposure and effects, and identify susceptible individuals (53).

CONCLUSION

This study of lead exposure has provided some data for risk assessment, such as in the rate of abnormalities in specific biomarkers of lead effect in individuals in lead production and refining versus unexposed controls. It also examined the relationship between occupational exposure and lead toxicity, focusing on haematopoiesis. Our data confirmed the association between occupational lead exposure and abnormalities in specific biological markers of lead effect and the role of occupational exposure in the development of adverse effects. We also investigated the possible influence of smoking, alcohol consumption, and lead air pollution caused by car exhaust (since lead is still used as a petrol additive) and melting plant emissions. Our data corroborate the need to devise preventive measures and activities for exposed individuals, and to improve regulation of occupational and environmental exposure. As genetic polymorphism of ALAD has been confirmed to modify lead toxicity, identification of susceptible individuals and population groups should be integrated in regulatory and specific protective public health solutions.

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Sažetak

ŠTETNI UČINCI ANORGANSKOG OLOVA U METALURŠKIH RADNIKA

Napravljeno je retrospektivno ispitivanje koje je obuhvatilo 60 radnika profesionalno izloženih anorganskomu olovu te skupinu od 60 kontrolnih ispitanika. Ispitivanje se temeljilo na posebnome upitniku o izloženosti olovu i procjeni toksičnoga djelovanja, liječničkome pregledu, spirometriji, EKG-u te laboratorijskim pretragama za olovo u krvi i toksične učinke olova. Statistički značajno zastupljeniji simptomi zamijećeni u izloženih radnika bili su bol u mišićima i klonulost te teškoće s disanjem na nos povezane s poslom. Zastupljenost plućnih teškoća bila je nešto viša u radnika izloženih olovu, ali ne značajno (20 % naprema 6,6 %). Srednje razine olova i delta-aminolevulinske kiseline (d-ALA) u krvi bile su značajno više u izloženih radnika. Usto je i aktivnost enzima dehidrataze d-ALA (ALAD) bila značajno niža negoli u kontrolnih ispitanika. Učestaliji abnormalni nalazi olova, ALAD-a i d-ALA zamijećeni su u krvi radnika izloženih olovu, a statistička je značajnost zabilježena za olovo i ALAD. Inverzna je korelacija utvrđena između olova i ALAD-a u krvi, a pozitivna korelacija između olova u krvi i dobi, godina zaposlenja, godina izloženosti, krvnoga tlaka, konzumacije alkohola i godina konzumacije alkohola. Promjene u spirometrijskim parametrima inverzno su korelirale s olovom u krvi. Pozitivno su korelirali olovo u krvi i broj eritrocita te koncentracija hemoglobina, a inverzno ALAD i koncentracija hemoglobina. Značajne su razlike uočene za olovo u krvi odnosno ALAD s visokim omjerom izgleda (14.64 odnosno 7.23), a također za relativni rizik (4.18 odnosno 3.08). Naši podaci potvrđuju povezanost između profesionalne izloženosti olovu i abnormalnih nalaza specifičnih biopokazatelja djelovanja olova te upućuju na ulogu profesionalne izloženosti u nastanku štetnih učinaka.

KLJUČNE RIJEČI: *biopokazatelji, dehidrataza delta-aminolevulinske kiseline, izloženost, olovo u krvi, toksičnost*

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