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LEVEL OF DNA DAMAGE IN LEAD-EXPOSED WORKERS

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Abstract: Lead plays a significant role in modern industry. This metal is related to a broad range of physiological, biochemical and behavioural dysfunctions. The genotoxic effects of lead have been studied both in animals and humans in *in vitro* systems but results were contradictory. The aim of this study was to investigate the association between DNA damage and occupational exposure to lead in workers. The study population consisted of 62 employees of metalworks exposed to lead in the southern region of Poland. The control group consisted of 26 office workers with no history of occupational exposure to lead. The concentration of lead (PbB) and zincprotoporphyrin (ZPP) in blood samples were measured. The DNA damage was analyzed in blood lymphocytes using alkaline comet assay. The level of DNA damage was determined as the percentage of DNA in the tail, tail length and tail moment. The lead exposure indicators were significantly higher in lead exposed group: PbB about 8.5 times and ZPP 3.3 times. Also, the percentage of DNA in the tail (60.3±14 *vs.* 37.1±17.6), comet tail length (86.9±15.49 *vs.* 73.8±19.12) and TM (57.8±17.82 *vs.* 33.2±19.13) were significantly higher in the study group when compared with the controls; however, the difference between the subgroups was only 5–10%. Years of lead exposure positively correlated with all comet assay parameters (R=0.21–0.41). Both mean and current PbB and ZPP were correlated with tail DNA % and TM (R=0.32; R=0.33; R=0.24; R=0.26 and R=0.34; R=0.33; R=0.28 and R=0.28, respectively). This study shows that occupational exposure to lead is associated with DNA damage and confirmed that comet assay is a rapid, sensitive method suitable for biomonitoring studies.

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INTRODUCTION

Heavy metals exert a huge influence on the environment and people's health. One of the most abundant heavy metals in the Earth's crust is lead. Lead is usually found in ore with zinc, silver and (most abundantly) copper, and is extracted together with these metals. Lead has been commonly used for thousands of years because it is widespread, easy to extract and easy to work with. Due to its properties, such as corrosion resistance, density, low melting point and ductility lead and lead compounds play a significant role in modern industry, e.g. in the production of batteries, metal products (solder and pipes), ammunition, paints, gasoline [1].

Lead can be absorbed following inhalation, oral, and dermal exposure, but the latter is much less efficient than the former two. After absorption into the bloodstream most of the lead is carried, bound to erythrocytes. The freely

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diffusible plasma fraction is distributed extensively throughout tissues, reaching highest concentrations in bones, teeth, liver, lungs, kidneys, brain and spleen. Lead in blood has an estimated half-life of 35 days, in soft tissue 40 days and in bone 20–30 years. Organic lead compounds are actively metabolized in the liver by oxidative dealkylation by cytochrome P-450 enzymes. Metabolism of inorganic lead consists of the formation of complexes with a variety of protein and nonprotein ligands. Lead is excreted mainly by renal and gastrointestinal pathways. It is excreted quite slowly from the body, hence accumulation in the body occurs easily [1, 12].

The toxicity associated with lead is widely known; however, the biochemical and molecular mechanism of lead toxicity are poorly understood. The International Agency for Research on Cancer (IARC) lists inorganic lead compounds as a possible human carcinogen (2B group). Organolead compounds are classified in group 3, not classifiable as carcinogenic to humans [16]. This metal is related to a broad range of physiological, biochemical, behavioural dysfunctions; it can also lead to hypertension, developmental defects, neurological problems, renal dysfunction and anemia. Lead induces micronuclei [2, 30], DNA damage [7, 13, 24, 35] and significant increase both in chromosomal aberrations [2, 15] and sister chromatid exchange [2, 15, 31]. The genotoxic effects of lead have been studied both in animals and humans in *in vitro* systems but results were contradictory [5]. At high concentrations, lead binds to DNA and changes its conformation, induces cell proliferation and breaks down nucleic acids. Lead compounds may cause genetic damage through several indirect mechanisms such as inhibition of DNA synthesis and repair, oxidative damage, interaction with DNA-binding proteins [12].

DNA damage and repair appear to be modulated by interactions between environmental and genetic factors. Response to environmental factors often depends on specific genetic polymorphisms [10]. One of the most studied genes that can affect toxicity of lead is δ-aminolevulinic acid dehydratase (ALAD). ALAD is the second enzyme in the heme biosynthetic pathway and plays a role in the pathogenesis of lead poisoning. The ALAD gene exists in two polymorphic forms that may modify lead toxicokinetics and influence individual susceptibility to lead [21]. Individuals carrying one or two copies of ALAD-2 allele present higher blood levels than individuals with only the ALAD-1 form of the gene. The protective effect of ALAD-2 is connected with the tight binding of lead by ALAD-2 protein and maintaining lead in a less bioavailable form [21, 34]. DNA response to exposure can be also modulated by genetic polymorphism in metabolic genes, such as glutathione-S-transferase (GST). There are some studies which have shown an association between DNA damage and GST polymorphism [11, 27].

The systemic uptake of lead from different sources contributes to the total body burden of lead. Blood lead (PbB) level and zinc protoporphyrin (ZPP) are used as a measure or biomarker of environmental as well as occupational exposure to lead [25]. In recent years the alkaline version of the single cell gel electrophoresis (SCGE) has become a new tool in the area of genetic toxicology. Comet assay (SCGE) is capable of detecting single-strand DNA breaks (SSB), double-strand DNA breaks (DSB), alkali labile sites (apurinic/apyrimidic sites), crosslinks, incomplete DNA repair sites and DNA damage induced by reactive oxygen species [6, 18, 19, 33]. Different kinds of DNA damage are repaired by different repair pathways. Smaller lesions, such as oxidized or alkylated bases, are recognized by specific glycosylases at the initial stage of base excision repair (BER) [20]. Nucleotide excision repair (NER) is an another highly sophisticated DNA repair pathway, involved *inter alia* in repair of oxidized bases [9, 10].

The aim of this study was to investigate the association between DNA damage and occupational exposure to lead in workers.

MATERIALS AND METHODS

The study population consisted of 62 employees (male) of metalworks exposed to lead in the southern region of Poland. In order to determine the degree of exposure to lead compounds, the concentration of lead (PbB) and zinc-protoporphyrin (ZPP) in blood samples were measured. Workers had been exposed to lead for about 14 ± 10 years and the values of PbB and ZPP were higher those within normal ranges (PbB > 20 μg/dl or ZPP > 5 μg/dl). Workers suffering from malignant tumours, diabetes, serious liver, kidney or heart insufficiency were excluded. The examined population exposed to Pb was additionally divided into four subgroups based on mean blood lead level measured every 3 months during last two years ($PbB_{mean\ 2y}$):

- lead-exposed subgroup $PbB_{mean\ 2y}$=20–40 μg/dl (n=18)
- lead-exposed subgroup $PbB_{mean\ 2y}$≥40–45 μg/dl (n=14)
- lead-exposed subgroup $PbB_{mean\ 2y}$≥45–50 μg/dl (n=23)
- lead-exposed subgroup $PbB_{mean\ 2y}$≥50–60 μg/dl (n=7)

The control group consisted of 26 office workers with no history of occupational exposure to lead. They all presented normal PbB and ZPP levels (normal values of non-occupational exposure to lead not exceeded the PbB of 10 μg/dl and ZPP of 2.5 μg/dl). None of the controlled subjects had a history of abnormalities regarding the above parameters. Only environmental exposure to lead occurred in the group controlled.

Blood samples (10 ml) were collected by venipuncture into 10-ml sterile tubes containing ethylenediaminetetraacetic acid (EDTA) solution as an anticoagulant.

Evaluation of lead intoxication parameters. In the whole blood, PbB and ZPP were determined. Analysis of lead in blood (PbB) was undertaken by graphite furnace atomic absorption spectro-photometry using Unicam 929 and 939OZ Atomic Absorption Spectrometers with GF90

and GF90Z Graphite Furnaces. Data are provided in µg/dl. Concentration of zinc protoporphyrin in blood (ZPP) was assayed directly using Aviv Biomedical haemato-fluorometer model 206 which measured the ratio of fluorescence of ZPP to absorption of the light by sample (by haemoglobin) and is presented as µg ZPP/g of haemoglobin (µg/g Hb).

Lymphocytes isolation. The remaining blood was centrifuged and lymphocytes were isolated. The isolation of lymphocytes from whole blood was performed by the gradient density centrifugation method using Histopaque solution 1077 (Sigma). 4 ml of whole blood was carefully layered on top of 4 ml of Histopaque 1077 and then centrifuged for 30 minutes. The lymphocytes were collected (about 1 ml) from the intermediate zone between Histopaque and plasma. The lymphocytes were then washed in a 0.9% NaCl and centrifuged. The 900 µl of serum (GIBKO) and 100 µl of DMSO medium was added to the cell pellet and mixed. The cell suspension was stored at -80ºC.

Comet assay method. The DNA damage was analyzed in blood lymphocytes using alkaline comet assay according to the method by Singh *et al.* [26] with some modification. Slides were prepared in duplicate per person.

A 100 µl of suspension of lymphocytes and 1% low melting point agarose (Sigma) was placed on a microscope slide that had been pre-coated with 0.5% normal melting point agarose (Sigma). Coverslips were placed on the gels, which were left to set on ice. After gently removing the coverslips, the slides were immediately submersed in lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Tritron X-100, pH 10) at 4ºC for 1 hour in the dark. The slides were then washed with cold PBS solution.

Afterwards, the slides were placed in a horizontal electrophoresis tank filled with electrophoresis buffer (300 mM NaOH, 1mM EDTA) for 40 minutes at 4ºC to DNA unwinding and denaturation. Electrophoresis was carried out for 30 minutes at 1.2 V/cm. All steps were performed in red light to reduce additional light-induced DNA damage.

After electrophoresis the slides were washed three times with a neutralization buffer (0.4 M Tris-HCl, ph 7.5), dried and stained with DAPI (4,6-diamidino-2-phenylindole) solution (1 µg/ml). The slides were stored in a closed humid chamber at 4ºC for 20 hours.

Slides were then analyzed by image analysis system Comet v. 5.5 (Kinetic Imaging Ltd., Liverpool, UK). To quantify DNA damage, the following comet parameters were evaluated: percentage of DNA in the tail (tail DNA %; relative fluorescence intensity of tail), tail length (distance from head centre to the end of the tail; in µm) and tail moment (TM) which was calculated as tail length x percentage of DNA in tail (in arbitrary units).

Statistical analysis. Statistical analysis was performed using Statistica 8.0 PL software. Statistical methods included mean, standard deviation (SD), standard error of mean (SEM). Shapiro-Wilk's test was used to verify normality and Levene's test to verify homogeneity of variances. An analysis of variance or Kruskal-Wallis ANOVA test was used for multiple comparisons of data. Additional statistical comparisons were made by t-test, t-test with separate variance estimates or Mann-Whitney U test. Spearman non-parametric correlation and regression analysis were calculated. A value of $p<0.05$ was considered to be significant.

RESULTS

DNA strand breaks were measured by comet assay in lymphocytes of lead exposed workers and controls. In this study, three parameters characterizing DNA strand breaks were evaluated: percentage of DNA in the tail, tail length and tail moment. Table 1 presents the characteristics of exposed and control groups. No statistical differences in age and body mass index (BMI) were found between lead exposed and control groups.

The percentage of smokers in the control group were 42%, while in the lead exposed group 60%, however, the difference was not statistically significant ($p>0.05$). In the lead exposed group, smokers comprised 56% of workers in the subgroup exposed to PbB = 20–40 µg/dl, 36% of workers in the subgroup exposed to PbB ≥ 40–45 µg/dl, 74% of workers in the subgroup exposed to PbB ≥ 45–50 µg/dl, and 71% of workers in the subgroup exposed to PbB ≥ 50–60 µg/dl (ANOVA $p=0.097$).

Lead exposure indicators were significantly higher in the lead exposed group: PbB about 8.5 times and ZPP 3.3 times. Also, the percentage of DNA in the tail (60.3 ± 14 *vs.* 37.1 ± 17.6), comet tail length (86.9 ± 15.49 *vs.* 73.8 ± 19.12) and TM (57.8 ± 17.82 *vs.* 33.2 ± 19.13) were significantly higher in study group when compared with the controls.

Table 1. Epidemiologic parameters, the blood lead level (PbB) and zinc protoporphyrin concentration in blood (ZPP) in study population.

	Control (n = 26)		Exposed (n = 62)		p
	mean	SD	mean	SD	
age (years)	40.4	10.4	39.2	10.3	0.601
years of lead exposure	–	–	14.2	9.97	–
years of non-lead exposure	–	–	25.0	6.05	–
BMI (kg/m^2)	25.63	3.49	26.5	4.14	0.371
$PbB_{mean\ 2y}$ (µg/dl)	5.41	2.27	43.17	7.89	<0.001
$PbB_{current}$ (µg/dl)	5.41	2.27	45.76	8.61	<0.001
$ZPP_{mean\ 2y}$ (µg/g Hb)	2.11	0.48	7.26	3.40	<0.001
$ZPP_{current}$ (µg/g Hb)	2.11	0.48	6.98	3.56	<0.001

BMI – body mass index, $PbB_{mean\ 2y}$ – mean blood lead level measured every 3 months during last two years, $PbB_{current}$ – current blood lead level, $ZPP_{mean\ 2y}$ – mean blood zincprotoporphirin measured every 3 months during last two years, $ZPP_{current}$ – current blood zincprotoporphirin.

Table 2. Correlation between studied parameters in study population.

	Tail DNA %	Tail length	TM
age (years)	NS	NS	NS
years of lead exposure	0.41***	0.21*	0.38***
years of non-lead exposure	-0.40***	-0.29**	-0.41***
BMI (kg/m^2)	NS	NS	NS
$PbB_{mean\ 2y}$ (μg/dl)	0.32**	0.22*	0.34**
$PbB_{current}$ (μg/dl)	0.33**	NS	0.33**
$ZPP_{mean\ 2y}$ (μg/g Hb)	0.24*	NS	0.28**
$ZPP_{current}$ (μg/g Hb)	0.26*	NS	0.28**

Spearman R values, *p<0.05; **p<0.01; ***p<0.001; NS – non significant.

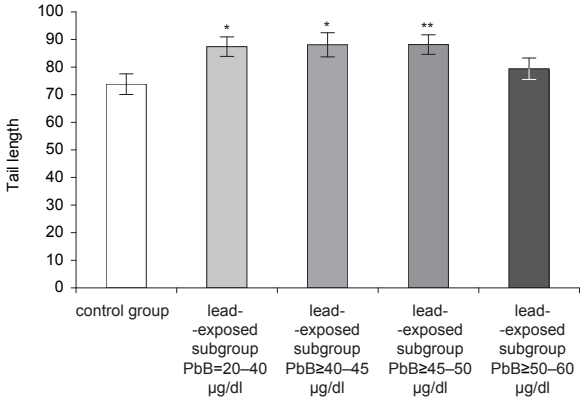

Figure 2. Level of tail length in lead exposed subgroups (*p<0.05, **p<0.01, ***p<0.001 compared to control).

Although the percentage of DNA in comet tail differed only within the range of 5–10% between lead-exposed subgroups (Fig. 1), it was significantly higher in each of this subgroup compared to control, as follows: 64.6±10.6 in PbB=20–40 μg/dl subgroup, 60.9±16.3 in PbB≥40–45 μg/dl subgroup, 56.7±14.9 in PbB≥45–50 μg/dl subgroup and 60.1±13.8 in subgroup PbB≥50–60 (ANOVA p<0.001).

The comet tail lengths were higher at 18% (87.4±14.9), 19% (88.1±16.4), 19% (88.2±16.9) and 8% (79.4±10.2) in lead-exposed subgroups, respectively (Fig. 2; ANOVA p=0.001). TM were higher at 84% (61.0±15.4), 81% (60.1±21.7), 67% (55.4±18.3) and 61% (53.4±14.9) in lead-exposed subgroups, respectively (Fig. 3; ANOVA p<0.001).

Correlations between the studied parameters are shown in Table 2. Years of lead exposure positively correlated with all comet assay parameters (R=0.41; R=0.21; R=0.38). Both mean and current PbB and ZPP levels were correlated with tail DNA % and TM. The analysis showed positive correlation between mean PbB level and tail DNA %, and mean PbB level and TM (R=0.32; R=0.34, respectively), as well as between current PbB level and tail DNA % (R=0.33) and current PbB level and TM (R=0.33). Positive correlation was also observed for mean ZPP levels and tail DNA % (R=0.24) and TM (R=0.28), as well as for current ZPP level and tail DNA % and TM (R=0.26; R=0.28, respectively). Regression analysis showed that only the concentration of Pb in blood ($PbB_{mean\ 2y}$) influenced to the percentage of DNA in the tail (R^2=0.25 p<0.001), comet tail length (R^2=0.08 p=0.007) and TM (R^2=0.20 p<0.001). The other parameters, such as age, time of lead exposure, smoking habits and BMI, did not affect comet assay parameters (p>0.05).

DISCUSSION

Environmental as well as occupational exposure to lead has become a major public health problem [29]. The genotoxic effect of lead in lymphocytes of the occupationally exposed group was examined by the single cell gel electrophoresis. Having a relatively long half-life, the lymphocytes are suitable for studying the effects of environmental or occupational exposures [13]. For the last decades, the comet assay has been used extensively to study genotoxic effects in human biomonitoring studies [17, 32, 33].

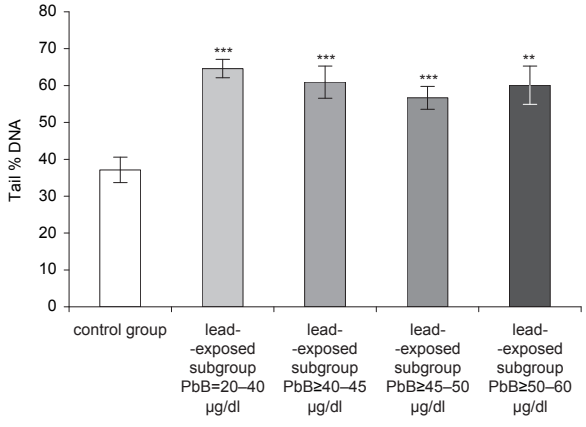

Figure 1. Level of tail % DNA in lead exposed subgroups (*p<0.05, **p<0.01, ***p<0.001 compared to control).

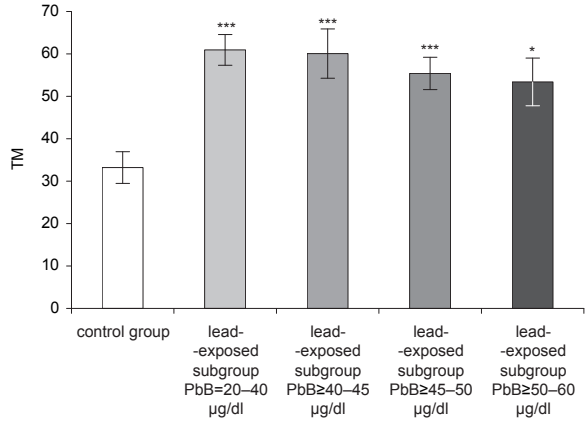

Figure 3. Level of tail moment in lead exposed subgroups (*p<0.05, **p<0.01, ***p<0.001 compared to control).

Our investigation revealed a statistically significant increase in the level of DNA damage in the exposed group compared to the controls. Occupational exposure in workers was associated with significantly higher levels of lead in blood concentration (PbB) and concentration of zinc protoporphyrin in blood (ZPP) in comparison with the unexposed group. Many other studies also indicated that workers exposed to Pb had significantly higher levels of DNA breaks measured with comet assay [3, 7, 13, 22, 24, 28]. There was a significant correlation between DNA damage (tail % DNA and tail moment) and all lead intoxication parameters, including PbB. However, the tail length positively correlated only with $PbB_{mean\ 2y}$. Tail length parameter was widely used in biomonitoring studies, although it has been criticized due to sensitivity to the background or threshold setting of the image analysis programme. The tail increases in intensity but not in length as the dose of damage increases [6, 33]. According to Valverde and Rojas [33], most of the biomonitoring studies found an induction of DNA damage as a result of occupational exposure to metals, although this has not been confirmed by anyone else [4, 8]. DNA damage was observed in a mice model of lead inhalation by Valverde *et al.* [31]. The level of DNA strand breaks was correlated with length of exposure and lead concentration in the tissue. The level of DNA damages significantly increased with increase in duration of exposure, and confirmed by other investigations [7].

The present study did not find any correlation between BMI, age and comet assay parameters. Many published studies did not detect an age-related increase in DNA damage [7, 13, 22, 28]. The age of an individual appeared to have little effect on the mean level of DNA damage, possibly due to decreased DNA repair capacity with age [3, 19].

Smoking is a well-known genotoxic and carcinogenic agent which induces an increased frequency of SCE and MN formation [23]. According to Piperakis *et al.* [23], smoking appeared to have a significant effect on basal DNA damage. A study of 150 middle-aged men showed a significantly higher level of oxidative DNA damage in smokers compared with non-smokers [11], although this was not confirmed in other studies [7, 13, 14, 22]. The lack of effect of smoking could be due to low statistical power. Meta-analysis of 38 studies indicated the association between smoking habits and levels of DNA damage [14].

This study shows that occupational exposure to lead is associated with DNA damage, and confirmed that comet assay is a rapid, sensitive method suitable for biomonitoring studies.

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