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Implications of oxidative stress in occupational exposure to lead on a cellular level

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Abstract

The aim of this study was to determine oxidative alterations leading to cellular dysfunctions in Pb exposed subjects by evaluating damage to all major classes of biomolecules in the cell, lipid peroxidation, protein and DNA damage and determine relationships between parameters of Pb toxicity and specific biomarkers of oxidative damage.

Analysis was conducted of smelter workers with high blood Pb and urine aminolevulinic acid levels and slightly elevated values of coproporphyrin and erythrocyte protoporphyrin IX. Significant decreases of thiol groups and increases in carbonyl groups as protein degradation end products, and of nitrite were detected. Elevated rates of lipid peroxidation and rises in the activities of the antioxidant enzymes Cu-Zn superoxide dismutase and catalase were also observed. Both enzymes showed positive correlations with the blood lead levels and urine coproporphyrin, while thiol groups correlated negatively with the same indices. The genotoxic potential of lead was manifested through an increased number of DNA damaged cells. Increased activities of serum lactate dehydrogenase isoenzymes indicated cellular damage in the lungs, kidneys, and liver. These lead induced impairments should be taken into consideration in assessment of Pb related health hazards.

Key words: lead (Pb), oxidative stress, DNA damage, protein oxidative modification, cell membrane damage

1. Introduction

Lead still poses a significant health hazard in occupationally exposed populations, especially in developing countries with heavy industry-based economies. Monitoring lead exposure is a most important measure in prevention of industrial lead poisoning and associated adverse health effects in workers (Ortega, Counter, and Jacobs 2013). Lead exposure elicits various physiological, biochemical, and genetic dysfunctions in laboratory animals and humans (Basgen and Sobin 2014; Flora, Mittal, and Mehta 2008; Sobin et al. 2013). Pb promotes oxidative stress directly through overproduction of reactive oxygen species (ROS), originating from interference with the heme synthetic pathway (Bechara et al. 1996; Jangid et al. 2012), but also indirectly via the simultaneous depletion of the cellular antioxidant pool (Ercal, Gurer-Orhan, and Aykin-Burns 2001; Gurer and Ercal 2000). When the cellular redox balance is disturbed and the antioxidant defense systems overwhelmed, cells are prone to oxidative damage and pathological conditions may occur (Rahman 2007). The synergistic effects of Pb-induced generation of ROS and inflammation were previously associated with disruption of cellular functioning leading to neuronal apoptosis (Suresh et al. 2012). Recent findings also indicate that exposed subjects exhibit long-term persistence of Pb-associated adverse effects and may have increased risks of developing late-onset diseases such as Alzheimer's disease (Behl et al. 2010; Jiang et al. 2008; Suresh et al. 2012). There are many reports of lead-induced pathophysiological changes in brain, heart, kidneys, and in reproductive organs that implicate oxidative stress in the mechanisms of toxicity (Ding, Vaziri, and Gonick 1998; Menke et al. 2006; Muntener et al. 2003; Schafer et al. 2005; Siddiqui, Srivastava, and Mehrotra 2002). Although lead intoxication has been broadly explored, the role of oxidative stress during the early-stages of adverse manifestations of Pb on a cellular level preceding clinical manifestations is still poorly understood.

The aim of this study was to determine the cellular sensitivity to oxidative stress in a population of workers exposed to lead by exploring the relationships between indicators of Pb exposure with a set of biomarkers of oxidative damage in all major classes of biomolecules of the cell.

Appropriate markers from peripheral blood lymphocytes and plasma were used, since the metabolism of the lymphocytes could reflect the state existing in cells of other tissues as well (Shive et al. 1986).

2. Materials and methods

2.1. Subjects

The tests were performed in 17 male subjects aged 35-55 years who were employees of a smelting plant (years of exposure 5.8 ± 4.8). The control group consisted of 10 healthy male volunteers matched in age, dietary habits, not exposed to lead compounds, in whom no increased PbB, EPP IX or CP concentrations and ALA-U were found. The use of biological material for this study was approved by the Ethical Commission of the Institute of Occupational Health of Serbia “Dr Dragomir Karajović” Belgrade, Serbia. No 03/2011.

2.2. Blood sampling

Blood samples for the tests were obtained from the participants in the morning, on empty stomach, by venipuncture from the *basilic* vein, in volumes of 14 mL, using heparin blood collection tubes (Greiner Bio-One, Kremsmünster, Austria). An aliquot of 10 mL was centrifuged for 10 min at 3000 rpm to obtain 4 mL of plasma. The sedimented erythrocytes were rinsed three times with 7 mL of physiological saline following each centrifugation. Both the

plasma and the erythrocytes were kept at -20 °C until analysis. From the remaining portion of 4 mL heparinised blood, lymphocytes were isolated with Ficoll-Paque medium (GE Healthcare Life Sciences, Pittsburgh, PA, USA) by centrifugation at 1900 rpm for 15 min, forming a layer directly above the medium. The lymphocytes were washed twice with 10 mL of RPMI 1640 medium (PAA Laboratories, Pasching, Austria), each wash followed by centrifugation for 10 min at 1800 rpm. Finally, the supernatant was removed by pipette without disturbing the pellet. The latter was resuspended in 1 mL RPMI 1640. The lymphocytes were checked for viability by Trypan blue exclusion method (Anderson et al.1994) and immediately used for the comet assay.

2.3. Biochemical assays

2.3.1. Oxidative stress parameters

The activity of Cu-Zn SOD (SOD1) in erythrocytes was determined by spectrophotometry (Misra and Fridovich 1972), that of catalase activity in erythrocytes by the method of Aebi (1984). Lipid peroxidation in erythrocytes was quantified by measuring the formation of thiobarbituric acid reactive substances (TBARS) (Stocks and Dormandy 1971) and expressed in nmol MDA per g Hb. The concentration of nitrite in plasma was determined with Griess reagent (Guevara et al. 1998) on a microplate reader at 540 nm (Mod. A1, Nubenco Enterprises, Paramus, NJ, USA). Results were shown in $\mu\text{mol L}^{-1}$. Isoenzymes forms of lactate dehydrogenase (LDH₁-LDH₅) were determined by means of vertical electrophoresis at 7.5 % PAGE (Hoeffer Mini VE, LKB, 2117, Bromma, Uppsala, Sweden) using tris-glycine buffer (25 mmol L⁻¹ tris, 192 mmol L⁻¹ glycine pH 8.3), sodium-lactate as substrate in the presence of nitro-blue tetrazolium chloride (Yoshida and Takakuwa 1997). After electrophoresis, gels were scanned (Hewlett Packard Scanjet 4850, CA, USA) and band intensity was measured by using

Scion Image 4.02 for Windows Software program package. The relative activities of isoenzymes were shown in percentages in relation to the total band intensity.

2.3.2. Parameters of oxidative modification of proteins

Determining of plasma carbonyl groups was performed spectrophotometrically with 2,4-dinitrophenylhydrazine at 365 nm (Levine et al. 1990). The concentrations of carbonyl groups was calculated on the basis of the absorption coefficient for this chromogen ($\alpha = 22000 \text{ L mol}^{-1} \text{ cm}^{-1}$), the results being shown in $\mu\text{mol L}^{-1}$. The free thiol groups were determined spectrophotometrically with 5,5-dithiobis-2-nitrobenzoic acid (Ellman 1959) at 412 nm. Concentration of thiol groups was measured on the basis of molar extinction coefficient of the chromogen ($\alpha = 14150 \text{ L mol}^{-1} \text{ cm}^{-1}$), while the obtained results were shown in $\mu\text{mol L}^{-1}$. All spectrophotometric measurements were performed with UV/VIS spectrophotometer (Cecil CE 2021, Cambridge, UK).

2.4. Indicators of lead toxicity

Blood lead was determined by atomic absorption spectroscopy (model 9200X, "Pye Unicam", Cambridge, UK) (Tietz and Aldrich 1987) and expressed as $\mu\text{mol L}^{-1}$. δ -Aminolevulinic acid and coproporphyrin in urine were measured (Tietz and Aldrich 1987) using a UV-spectrophotometer (model Stasar III, Gilford, Ohio, USA) and expressed in $\mu\text{mol L}^{-1}$. EPP IX was quantified by using hematofluorometry (model Hematofluor 2P, Buchler Instruments, Fort Lee, New Jersey, USA) (Tietz and Aldrich 1987).

2.5. Comet assay

The alkaline comet assay was performed as described by Singh et al. (1988). The comets were analyzed 15 minutes after staining at 100 \times magnification on a microscope (BX 50, Olympus Optical Co., GmbH, Hamburg, Germany), equipped with a mercury lamp HBO (50W, 516-560 nm, Zeiss, Jena, Germany). Evaluation of DNA damage was done according to Anderson et al.

(1994). A sample of 100 randomly selected cells per subject, 50 cells from each of 2 replicate slides (to minimize possible differences resulting from position effects of slides during electrophoresis) were visually scored and classified into five categories corresponding to the amount of DNA released from the core of the nucleus that migrated in the tail resembling a “comet”: (A) no damage, <5%; (B) low level damage, 5-20%; (C) medium level damage, 20-40%; (D) high level damage, 40-95%; (E) total damage, >95. Comets were randomly chosen, avoiding counting at the edges of slides and occasional apoptotic cells. Analysis was always carried out by the same, experienced person. As a measure of DNA damage, each subject was characterized for the total number of cells with DNA migration over 5% (the sum of the comet classes B+C+D+E), and mean value (\pm standard deviation) for the total number of DNA damaged cells was calculated for all 17 subjects.

2.6. Statistical analysis

Statistical significance of differences for all examined parameters between controls and lead-exposed participants was determined by means of the unpaired Students' *t*-test. Data were expressed as means \pm standard deviation (SD). Spearman's correlation and linear regression were used to evaluate the relationships between parameters. Significance level was set at $p < 0.05$. Statistical analysis was performed using the Graph Pad Prism 5.0 Software, CA, USA.

3. Results

The impact of Pb on the antioxidant defense system was assessed through monitoring the activities of the radical scavenging enzymes superoxide dismutase (SOD) and catalase (CAT). Oxidative damage was evaluated by measuring the levels of malondialdehyde (MDA), as well as

by determining the concentrations of plasma protein thiols (SH), carbonyl groups (CG) as indicators of oxidative protein modification, and nitrite (NO_2^-). Alkaline single cell gel electrophoresis (comet assay) was applied for measuring oxidative DNA damage in peripheral blood lymphocytes, as it is a rapid and sensitive method that detects non-repaired primary lesions (Garaj-Vrhovac and Zeljezic 2000). The distribution of tissue specific lactate dehydrogenase (LDH) isoenzymes was used as an indicator of damage to the myocardial and red blood cells (LDH_1), white blood cells (LDH_2), lung (LDH_3), kidney and pancreas (LDH_4), as well as liver and skeletal muscle cells (LDH_5). The indicators used for the determination of the extent of lead toxicity were lead concentration in blood (PbB) and the following effect biomarkers: erythrocyte protoporphyrin-IX (EPP IX), urine coproporphyrin (CP) and delta-aminolevulinic acid in urine (ALA-U) (Jangid et al. 2012).

[Table 1]

The indicators of toxicity in subjects occupationally exposed to lead are referred to demographic characteristics of the study population that could affect the results of analyses, such as age, smoking and alcohol consumption habits as well as duration of employment and years of occupational exposure (Table 1). Levels of Pb in blood ranged from 0.4 to $3.2 \mu\text{mol L}^{-1}$, with a mean value of $1.7 \pm 0.9 \mu\text{mol L}^{-1}$. PbB concentration of $1.9 \mu\text{mol L}^{-1}$ (the workplace intervention limit), was exceeded in 47% of exposed study population. EPP IX and CP calculated means remained low according to the range for occupationally exposed populations. Urine δ -ALA showed high values $84 \pm 23 \mu\text{mol L}^{-1}$ compared to both general population and occupationally exposed population limit values. The duration of employment under conditions of Pb exposure ranged from 2 to 16 years (5.8 ± 4.8 years). Multivariate analyses were employed to evaluate the possible associations between the toxicological indices and demographic and lifestyle factors. No

significant correlation was found between the duration of either employment or professional exposure with the PbB ($r = 0.246$, $p < 0.378$; $r = 0.264$, $p < 0.341$; respectively) and other parameters of toxicity. Also, age and lifestyle habits did not correlate with any of the parameters monitored in this study (not presented in figures and tables).

[Figure 1]

Both SOD1 and CAT showed elevated activities in the exposure group ($p < 0.01$, $p < 0.001$) compared to controls (Figure 1).

[Figure 2]

Significant differences were also observed among the two groups in markers of oxidative protein modification, plasma thiols, carbonyl groups and nitrite (Figure 2). Significant increases were found in the carbonyl ($p < 0.0274$) and nitrite concentrations ($p < 0.0006$), while the contents of SH groups were decreased in workers compared to those in controls ($p < 0.001$) (Figure 2).

[Figure 3] [Figure 4]

Also, damage to nuclear DNA, evaluated through the mean number of cells with fragmented DNA, was significantly increased ($p < 0.001$) in lymphocytes of workers 23 ± 14 compared to the value found in control samples 7.7 ± 3.4 (Figure 3). While control samples displayed mostly intact, round nuclei with the DNA contained within the nucleus, samples of workers showed nuclei with different amounts of fragmented DNA resembling comets (Figure 4A, B).

[Figure 5]

When level of lipid peroxidation was quantified by MDA concentration, a significant rise in the group of exposed ($p < 0.001$) was revealed (Figure 5). Lactate dehydrogenase isoenzymes (LDH₁-LDH₅) activities and isoenzymatic distributions (Figure 6) show that the exposure group

had an increase in cellular damage versus controls. There were variations in results on isoenzymatic distributions among two groups, where the LDH₃, LDH₄ and LDH₅ showed significant rise in the exposure groups, indicating an increased degree of cell damage in the lungs (LDH₃), kidneys (LDH₄) and liver (LDH₅) of workers compared to controls.

[Figure 6]

Association between indices of toxicity and selected oxidative stress biomarkers in exposed population was evaluated (Figure 7). Positive correlations were found between antioxidant enzyme activities and two indicators of lead toxicity, i.e. the levels of PbB and CP, while SH groups showed negative correlations with the same parameters. CAT activity strongly correlated with PbB levels ($r = 0.898$; $p < 0.001$) and CP concentrations as well ($r = 0.704$; $p < 0.01$), while SOD1 displayed moderate correlation with PbB ($r = 0.586$; $p < 0.01$) and CP ($r = 0.527$; $p < 0.05$).

[Figure 7]

Linear regression analysis showed that increases in SOD1 and CAT activities followed linearly the rise in PbB concentrations and the increase in CP concentrations (Figure 7). Also, a significant negative correlation was found between the levels of SH groups and both indices of lead toxicity, PbB concentration and CP levels ($r = -0.898$; $r = -0.748$; respectively), where the trend in the SH group concentrations decrease fell linearly with the rise of PbB and CP concentrations (Figure 7).

4. Discussion

Different aspects of cellular dysfunction in Pb exposure still remain unknown and oxidative stress has been implicated as contributing factor (Ahamed and Siddiqui 2007). The focal aim of

present study was to examine associations of specific biomarkers of lead exposure and oxidative damage in all main types biomolecules in cells, proteins, lipids and DNA, and the existence of possible trend that could reflect the overall impact of Pb burden on oxidative changes in exposed group. Results in this study have shown an increase in the concentration of Pb and all biomarkers of effect in blood of exposed workers . While PbB is a useful indicator of acute and recent lead exposure, and could remain elevated for weeks, EPP IX could better reflect the chronic effects of lead toxicity since it rises slowly once PbB exceeds $1.5 - 2.0 \mu\text{mol L}^{-1}$ and remains elevated for several months (Froom et al. 1998; Martin, Werntz, and Ducatman 2004; Pagliuca et al. 1990). EPP IX can also be used as indicator of increased body lead burden (Froom et al. 1998). The elevated mean values of PbB and slightly increased EPP IX and CP found in this study group could be suggestive of recent exposure. These findings are consistent with other reports of acute lead poisoning that gave a same relation of parameters (Martin, Werntz, and Ducatman 2004; Pagliuca et al. 1990). This is in accordance with relatively short average duration of exposure reported in the exposed group (5.8 ± 4.8 years), although there was no correlation found between years of exposure nor employment with any of the toxicity indices.

Disruption of the heme synthesis pathway is one of the first adverse effects of lead toxicity (Ahamed and Siddiqui 2007). Accumulation of EPP IX and CP reflects the disturbance of the final step in heme synthesis, suggesting biochemical effect of active deposits of lead in bone marrow tissue (Sakai et al. 2000). ALA-U was also previously reported to be a valuable measure of metabolically active lead (Molina-Ballesteros et al. 1978). We determined high levels of ALA-U in exposed group, which could contribute to an oxidative stress increase through alterations in the heme synthesis pathway. Accumulated ALA acts as an endogenous pro-oxidant in Pb poisoning, promoting reactive oxygen species generation (Bechara 1996; Duydu and Suzen

2003; Grover et al. 2010). The prime targets of lead induced oxidative attack are biomolecules, such as lipid membranes, proteins and DNA and enzymes of antioxidant defense system (Adegbesan 2007; Bokara et al. 2008; Ercal, Gurer-Orhan, and Aykin-Burns 2001).

Results presented in this paper showed increase in the parameters of oxidative stress and damage to all major biomolecular components of the cells in lead-exposed workers. In addition, it was demonstrated that the alterations in antioxidant enzyme system correlated well with the indicators of toxicity, showing the effect of lead exposure on antioxidant system was concentration dependent.

Ariza, Bijur and Williams (1998) showed that a quick formation of cellular H_2O_2 occurred after treatment of AS52 cells with 1 mol L^{-1} lead which was followed by elevated activity of antioxidant enzymes. Increased levels of erythrocyte SOD1 and CAT found in this study group indicated that there was an increase in the level of ROS following lead exposure. Further, we found correlations between both antioxidant enzymes activity and thiol groups with the indicators of toxicity. On the other hand, Chiba et al. (1996) has found that although elevated, SOD did not show correlation with lead exposure, while CAT levels were markedly increased with the rising levels of PbB. Based on its strong correlation with indices of toxicity, CAT could be proposed as a potential biomarker in preventive monitoring of populations exposed to Pb. Carbonyl groups levels are most commonly used markers of protein oxidative modification (Stadtman and Levine 2000). They are found in high levels in number human diseases (Dalle-Donne et al. 2003). Significant decrease in concentrations of SH groups and increase in the levels of carbonyl groups and nitrite found in workers but not in matched controls were indication that protein damage resulted from lead exposure. Lead is known to bind to the functional sulfhydryl groups of enzymes, further contributing to the impairment of normal

oxidative balance in cells (Froom et al. 1998). Linear concentration-dependant relation between decrease in concentrations of SH groups and the increase in blood lead concentration in exposed workers found in this study, supports this notion. DNA strand breakage in peripheral blood lymphocytes by using comet assay was measured as parameter of DNA damage. Since DNA is most susceptible to oxidative stress, the level of DNA damage can be used as a potential biomarker of health outcome. Although lead has been previously tested for genotoxic potential in a wide range of assays, results obtained with different methods and model systems have been conflicting (Carmona, Creus, and Marcos 2011; Grover et al. 2010; Minozzo et al. 2004). Some works reported that Pb compounds failed to induce SCE or DNA single strand breaks in cell cultures, such as study of Zelikoff et al. (1988), while other studies on exposed animals and humans showed that lead can cause DNA damage and clastogenic and aneugenic effects (Devi et al. 2000; Kašuba et al. 2010; Palus et al. 2003). The examination of DNA damage in lymphocytes of subjects in this study showed that DNA integrity was affected in the group of workers exposed to Pb compared to unexposed controls, implying that genotoxic events occurred following lead exposure. This is in accordance with previously reported DNA damage found in lymphocytes of occupationally exposed individuals (Danadevi et al. 2003; Grover et al. 2010; Manikantan, Balachandar, and Sasikala 2010). Douki et al. (1998a) demonstrated on isolated DNA that the δ -ALA acts as an effective alkylating agent. Same authors reported that chronic treatment with δ -ALA increased the levels of 8-oxo-7,8-dihydro-2-deoxyguanosine in rats DNA, further supporting genotoxic potential of δ -ALA (Douki et al. 1998b). The studies on genetic effects of Pb in exposed humans are controversial, but considering the above mentioned it seems plausible that the genotoxicity of lead is based on indirect mechanisms, most likely as a consequence of δ -ALA accumulation, rather than causing alterations in DNA directly. However,

increased levels of DNA damage found in exposed workers in this study supports previous findings on association of Pb exposure and genotoxicity and deserves further explorations. Lead is known to have damaging effects on lipid structures as well which may affect membrane integrity, permeability, and function (Lawton and Donaldson 1991; Yiin and Lin 1995). Raised level of MDA found in workers in this study supports previous reports of increased lipid peroxidation following lead exposure (Ito et al. 1985; Yiin and Lin 1995). Peroxidation degradation products (MDA) lead to cross-linking reactions between proteins and lipids in the membrane, decreasing its fluidity and causing consequential impairment of its functions (Levin, Cogan, and Mokady 1990). Peroxidation process is an important indication of membrane damage that ultimately can promote cell death or necrosis (Ohyashiki, Sakata, and Matsui 1992). Cytoplasmic enzyme lactate dehydrogenase that leaks from injured cells has shown to be a useful indicator of cellular membrane damage (Neal et al. 1997). Watson, MacDonald-Wicks, and Garg (2005) showed that the activity of lactate dehydrogenase is increased in the presence of hydroxyl radicals. Previous reports on cultured cells exposed to Pb also demonstrated rise in LDH release due to oxidative interactions *in vitro* (Sanders, Liu, and Tchounwou 2014; Suresh et al. 2012). The increased activity of LDH found in plasma of workers in this study were probably an outcome of cellular membrane destruction due to increased peroxidation. The distribution of LDH isoenzymes can indicate the potential damage to certain organs (Jović et al. 2013), according to their tissue specificity. The revealed LDH₁₋₅ isoenzyme distribution showed variations in results among two groups, depending on the degree of oxidative damage in each tissue. Higher levels of LDH₃, LDH₄ and LDH₅ in exposed group, originating from the lung, kidney and liver, point toward assumption that these tissues were most affected by oxidative damage. To the best of our knowledge, this is the first study in which the origin of tissue-specific

cell damage induced by lead *in vivo* through LDH indicators is shown. These findings are in accordance with the fact that exposed subjects worked in the smelting facility at which there was a direct respiratory exposure to Pb fumes. The biggest cell damage was detected in the lungs, which is most probably an outcome of direct inhalation of vapours containing Pb.

Nephrotoxicity and hepatotoxicity are also very common hallmarks of lead exposure (Wang et al. 2002). Fontanellas et al. (2002) has shown that the ratio of erythrocyte ALAD can be responsible for renal impairment. High ALA-U content measured in the exposed group could have promoted cell damage in kidneys by above described molecular oxidative process (Bechara 1996; Hermes-Lima et al., 1991). Still, it is not clear whether lead exposure or the resultant increase in ROS is the cause of this damage.

5. Conclusions:

Based on the data presented, oxidative stress appears to be a plausible mechanism of causing cellular dysfunction in workers exposed to toxic levels of lead. The present study provided evidence of lead induced oxidative damage and that the alterations in the antioxidant defense system is related to occupational lead exposure. Genotoxic effects of lead were also demonstrated, as were indices of cellular damage in liver, kidneys and lungs associated with Pb exposure.

Although this is only a pilot study with a limited number of participants, results found in our study group might reflect adverse effects for lead exposed populations and indicate increased risk of pathologies linked with oxidative impairment. These findings require studies that would provide further explanations on links between long-term effects of these Pb-induced cellular dysfunctions and increased risk of developing late-onset diseases.

Conflict of interest

We declare that we have no financial or non-financial competing interests.

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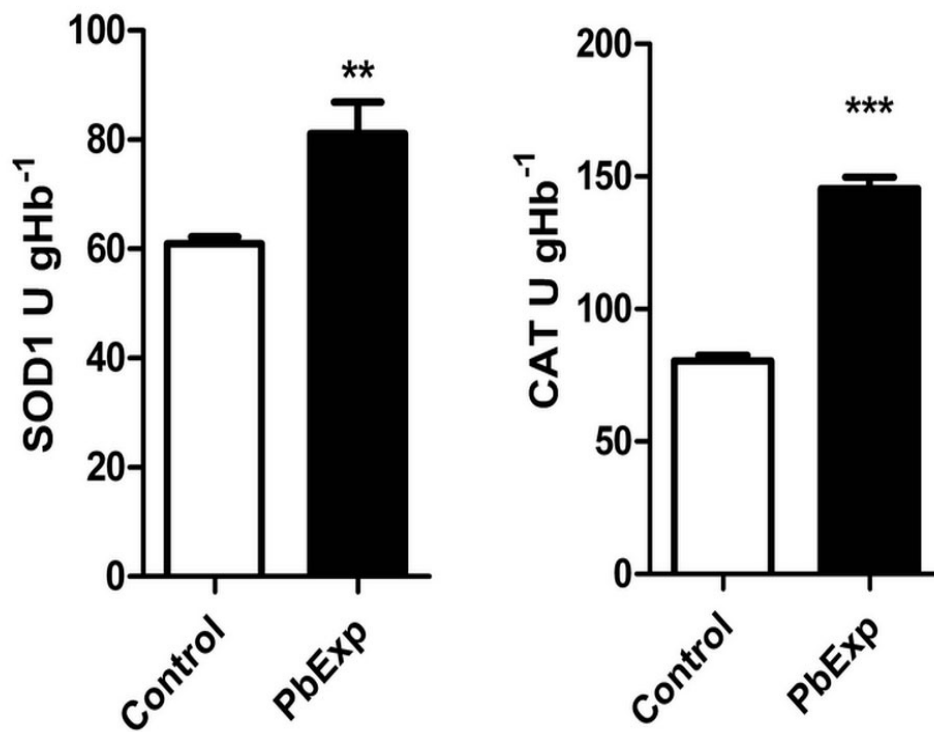


Figure 1: Activity of antioxidant enzymes Cu-Zn superoxide dismutase (SOD1) and catalase (CAT) in exposed workers (PbExp, n = 17) vs. controls (Control, n = 10). Bars represent mean values \pm SEM, **p < 0.01, ***p < 0.001, by using t- test.

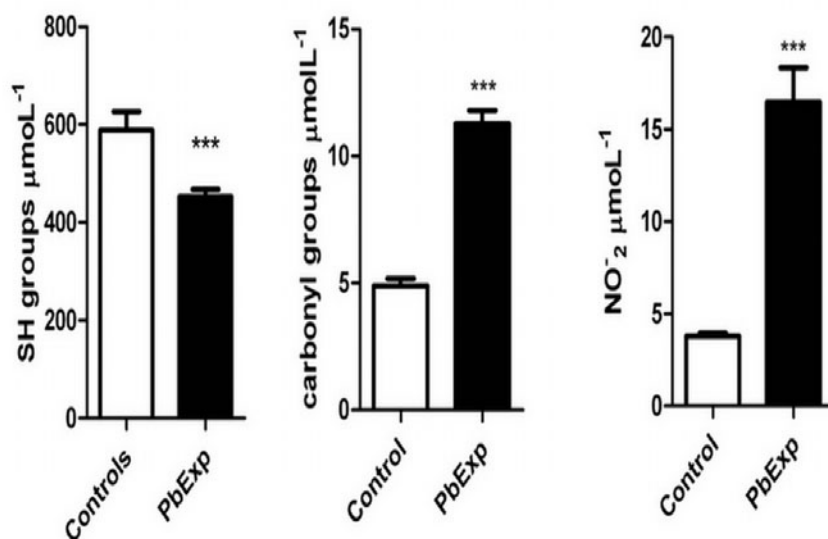


Figure 2: Levels of plasma thiols (SH), carbonyl groups (CG) and nitrites (NO₂⁻) as markers of protein oxidative modification found in exposed workers (PbExp, n = 17) vs. controls (Control, n = 10). Bars represent mean values ± SEM, ***p < 0.001, by using t- test.

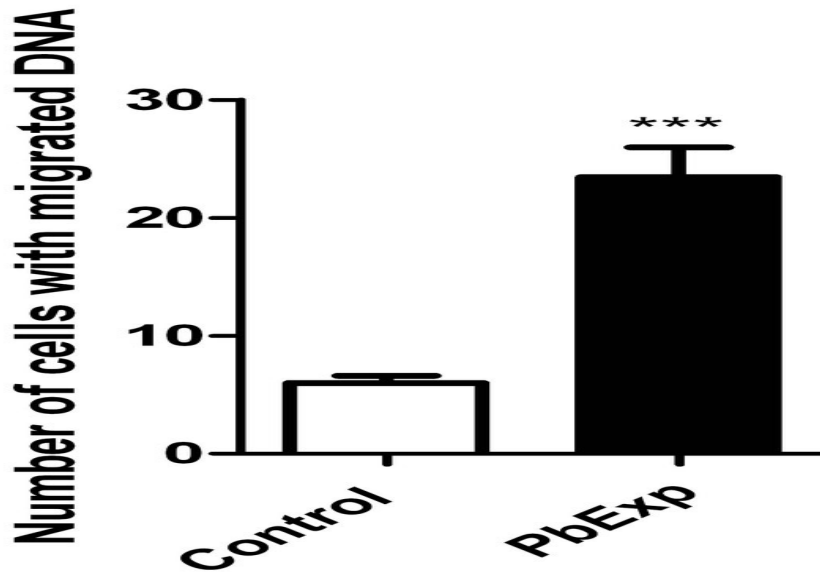


Figure 3: Number of cells with damaged DNA in exposed workers (PbExp, n =17) vs. controls (Control, n = 10). Bars represent mean values \pm SEM, ***p < 0.001, by using t- test.

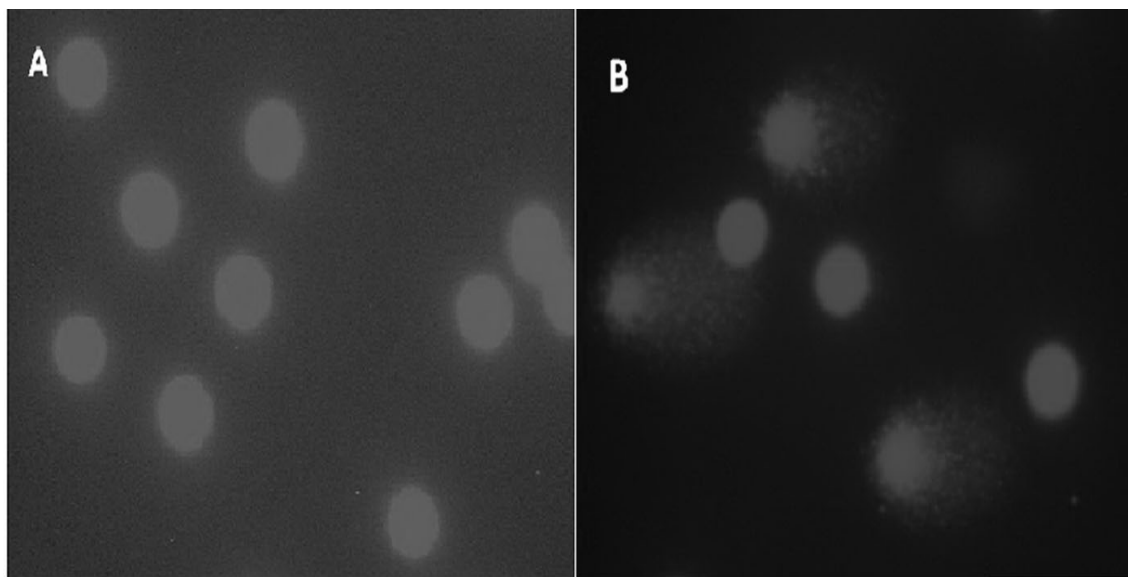


Figure 4: Evaluation of DNA damage by comet assay in lymphocytes of exposed workers and controls. Images of comets from (A) controls and (B) exposed subjects.

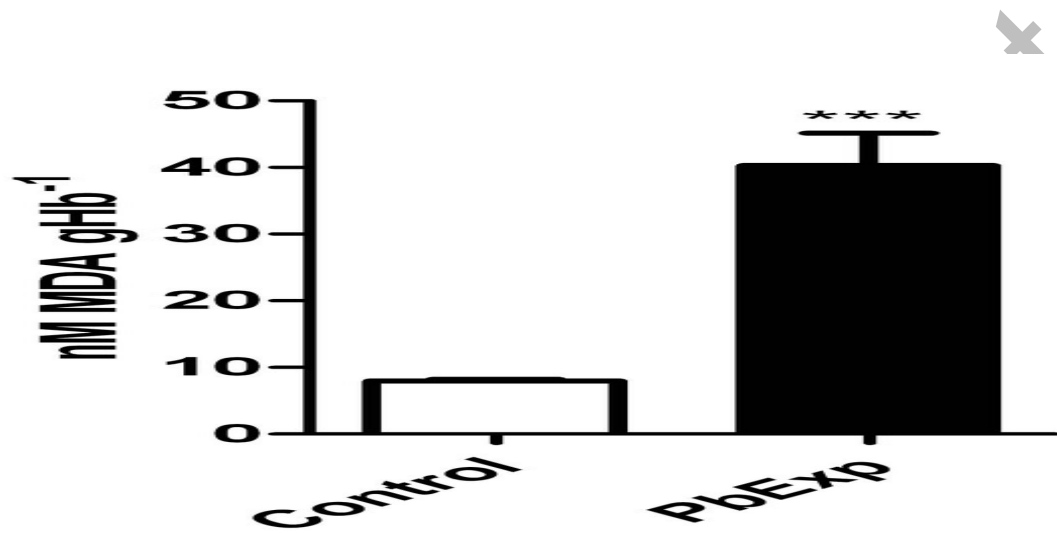


Figure 5: Level of malondialdehyde (MDA) as lipid peroxidation indicator in exposed workers. Bars represent mean values \pm SEM, *** $p < 0.001$, by using t- test.

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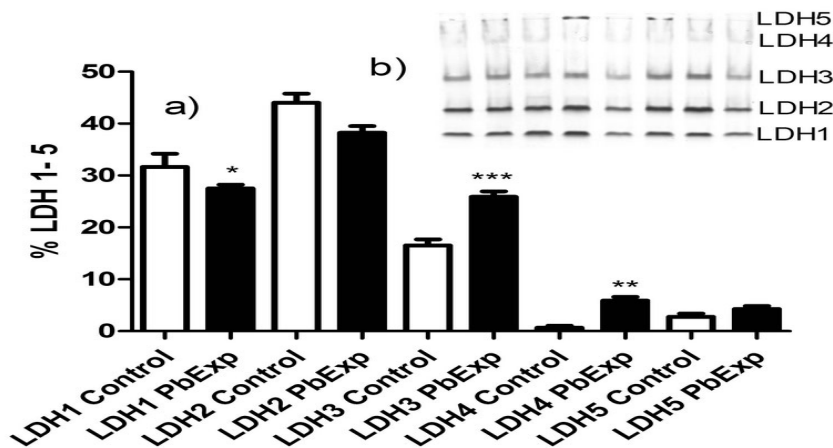


Figure 6: Distribution of tissue specific lactate dehydrogenase isoenzymes (LDH 1-5) found in exposed workers (PbExp, n = 17) vs. controls (Control, n = 10). A) LDH distribution expressed as percentage of the LDH content. Bars represent mean values from all subjects \pm SEM, *p < 0.05, **p < 0.01, ***p < 0.001, by using t- test. B) Native gel electrophoresis and activity staining of LDH isozymes; 1-control sample, 2-8 samples of exposed.

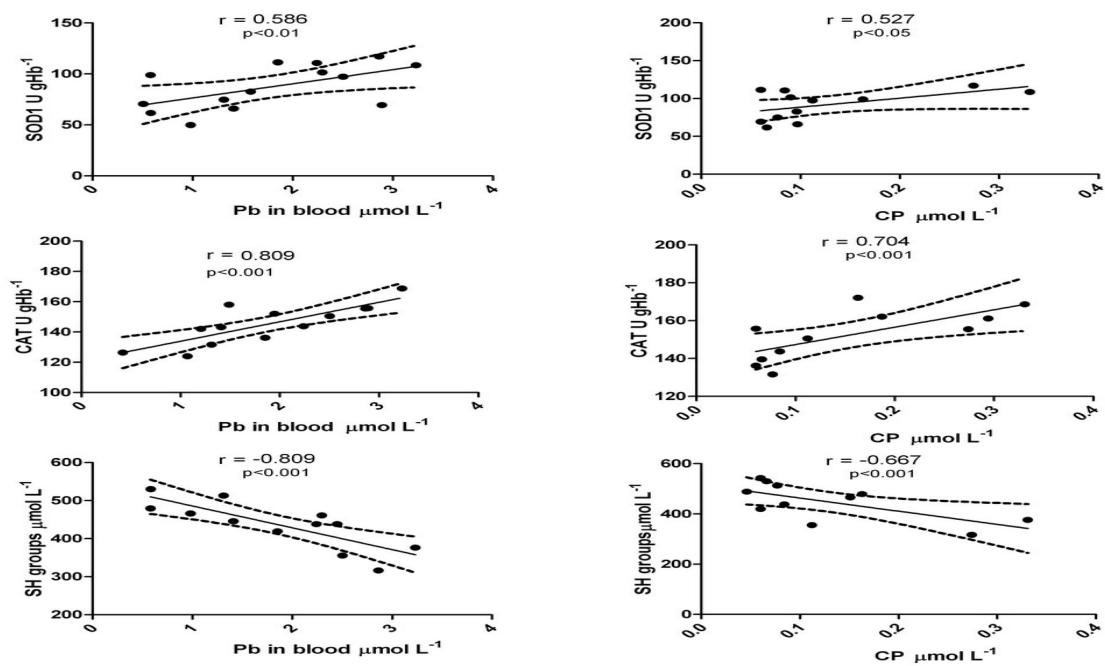


Figure 7: Significant correlations between Pb in blood (PbB) and Urine coproporphyrin (CP) with levels of Cu-Zn superoxide dismutase (SOD1), catalase (CAT) and plasma protein thiols (SH groups) were found in exposed subjects (n = 17).

Table 1 Demographic characteristics and selected clinical indices of lead toxicity in exposed subjects (n = 17) and controls (n = 10)

Parameters	Range PbExp	Mean \pm SD PbExp	Range for occupational exposure	No. of PbExp subjects	Mean \pm SD Controls	Control range	No. of Controls
Age (years)	35-55	46 \pm 9	-	17	31 - 53	44 \pm 9	10
Smoking (cigarettes per day)	1-5	3.4 \pm 1.3	-	7	1 - 3	23 \pm 0.8	5
Alcohol consumption (drinks containing alcohol per day)	1-4	2.0 \pm 1.2	-	12	1 - 2	0.5 \pm 0.8	4
Duration of employment (years)	5-39	22 \pm 11	-	15	0	0	0
Years of occupational exposure	2-16	5.8 \pm 4.8	-	15	0	0	0
Pb in blood (PbB) $\mu\text{mol L}^{-1}$	0.4-3.2	1.7 \pm 0.9	< 2.88 > 1.93 (intervention limit) < 1.93	17 8 9	0.21 \pm 0.02	< 0.48	10
Erythrocyte protoporphirin - IX (EPP IX) $\mu\text{mol L}^{-1}$	0.55-3.15	1.3 \pm 0.6	< 1.50	15	0.33 \pm 0.17	< 0.72	0
Urine coproporphirin (CP) $\mu\text{mol L}^{-1}$	0.05-0.33	0.14 \pm 0.08	< 0.280	15	0.03 \pm 0.01	< 0.17	10
δ -Aminolevulinic acid in urine (ALA-U) $\mu\text{mol L}^{-1}$	52 - 120	84 \pm 23	< 76.3	15	28 \pm 9	< 50	10