

## Original Paper

# Blood Lead Levels and Oxidative Stress

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## REZUMAT

### *Nivelurile de plumb din sânge și stresul oxidativ*

**Obiective:** Există din ce în ce mai multe dovezi cu privire la implicarea mecanismelor stresului oxidativ în intoxicația cu plumb. Scopul cercetării actuale a fost de a evalua posibilele asocieri între markerii stresului oxidativ la pacienții intoxicați cu plumb.

**Metodologie:** A fost selectat un lot de 111 pacienți diagnosticați cu intoxicație cu plumb din cadrul Clinicii de ATI - Toxicologie a Spitalului Clinic de Urgență București pentru care s-au efectuat determinări serologice ale nivelului de plumb, superoxid dismutază, glutatation peroxidaza și proteina C reactivă.

**Concluzii:** Rezultatele investigației efectuate au indicat creșteri ale superoxid dismutazei și proteinei C reactive proporțional cu plumbemia ( $p < 0.001$ ), în timp ce această corelație nu s-a putut realiza în ceea ce privește nivelul glutatation peroxidazei.

**Cuvinte cheie:** intoxicație cu plumb, superoxide dismutaza, stress oxidativ

## ABSTRACT

**Objectives:** More and more literature data sustain the implication of oxidative stress in relation to lead exposure. The aims of the present study were to assess the biochemical associations between lead exposure and oxidative stress.

**Methodology:** Blood from 111 patients presented for lead exposure in our Critical Care Toxicology Unit of Bucharest Clinical Emergency Hospital was analyzed in terms of lead level, superoxide dismutase (SOD), glutathione peroxidase (GP) and C-reactive protein (CRP).

**Conclusions:** Our present findings suggest that lead exposure is associated with higher values for SOD and CRP ( $p < 0.001$ ), whereas no statistical connection was established with GP level.

**Key words:** lead intoxication, superoxide dismutase, oxidative stress

## INTRODUCTION

Environmental lead sources are represented by air,

soil, food, water. Lead distribution in the environment continues to be a worrying public health issue considering its potential cumulative effects in the body due to various

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routes of exposure. Once exposed to lead, individuals have persistent elevated blood levels because of redistribution from bones sanctuaries, even years after cessation of exposure. Oxidative stress seems to play an important part regarding lead adverse effects, because the metal is responsible for reactive oxygen species (ROS) production and it also might inhibit antioxidants levels. Enzyme inhibition appears due to a strong affinity of lead for thiol groups; superoxide dismutase (SOD), catalase, glutathione peroxidase (GP) inactivity reduces the content of glutathione. Thiol groups are reducing agents with a concentration around 5 mM in animal cells. Out of these, SOD is located in the cytoplasm and nucleus, being a part of a larger enzyme family. Humans have 3 forms of SOD; SOD 1 in the cytoplasm, SOD 2 in the mitochondria and SOD 3 is extracellular. Their structure varies, the first one being a dimer and the other 2 being tetramers. Copper and zinc are part of SOD 1 and 3, while SOD contains manganese [1]. Its actions consist in alternately adding or removing an electron, converting into either hydrogen peroxide or ordinary molecular oxygen, less damaging species.

The glutathione (GSH) is the most abundant non-protein thiol group inside the cell, being a tripeptide of glutamic acid, glycine and cysteine. It has an important role in biochemical processes such as detoxification or metabolism regulating [2]. Chemically, glutathione reduces disulfide bonds existing within cytoplasm proteins to cysteine, serving as an electron donor. It becomes an oxidized form, known as GSSG. With the aid of NADPH as an electron donor, it can further be reduced by glutathione reductase [3]. GSSG scavenges reactive oxygen species and free radicals, enabling a protective effect against oxidative stress and toxins [4]. Medical practice pays much attention to GSH and GSH-reductase level in accordance to its clinical implications [5]. The enzyme is encoded by GSR gene in humans. It acts as a dimeric disulfide oxidoreductase and utilizes an FAD prosthetic group and NADPH to reduce one mole of GSSG to two moles of GSH. Oxidative balance of the cells depends upon the ratio of GSSH/GSH, mainly it is critical to maintain high levels of the reduced glutathione and low levels of oxidized glutathione disulfide [3]. Some other research areas regarding immunotoxicology pay attention of inflammatory potential of some heavy metals, including lead. The patterns of relationship between blood lead levels (BLLs) and C-reactive protein (CRP) is still not very well documented, although it has been stipulated that some of the lead toxic reaction in the organism have an inflammatory explanation.

#### Aims of the study

The aims of the present study were to assess the biochemical associations between lead exposure, oxidative stress and inflammatory status evaluated using SOD, GP and CRP.

## MATERIAL AND METHODS

This is a prospective study conducted in Critical Care Toxicology Unit of Bucharest Clinical Emergency Hospital; all patients presented for lead exposure were enrolled between 01.05.2013-01.02.2015. A total of 126 subjects from all over the country was the initial group. They all completed a self-administered questionnaire, were fully clinical evaluated and performed several blood tests on the admission day. Collected samples were then analyzed regarding lead plasma levels, SOD, GP, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and reactive protein C (CRP). Patients suffering from chronic illness like malignancies, hypertension, arthritis, and heart disease were excluded.

#### Lead determination

Lead was determined on admission day in blood obtained by venipuncture and immediately centrifuged for 15 minutes at 2000 rpm using atomic absorption spectrophotometry provided by Spectra AA-880. Final result was expressed in micrograms per deciliter and BLLs higher than 40 were considered as intoxication. Patients were divided in 5 classes of lead exposure as follows: Class I, BLL=10 to 14 µg/dL, Class II, BLL=15 to 19 µg/dL, Class III, BLL=20 to 44 µg/dL, Class IV, BLL=45 to 69 µg/dL, Class V, BLL>70 µg/dL.

#### Oxidative stress evaluation

Plasma biochemistry and antioxidant status was measured using refrigerated venous blood obtained on the admission day. The activity of SOD was measured in a standardized laboratory using photometric enzymatic method and expressed in Unit/g of Hb, within 1200-1600 as normal ranges [6]. It usually inhibits the auto-oxidation of pyrogallol and one unit is considered the amount of SOD needed for inhibition of 50% of pyrogallol auto-oxidation [7]. GP was analyzed using the same method in the same laboratory having 27.5-73.6 U/g of Hb as cut off values [6].

#### Biochemical parameters

The plasma constituents analyzed were alanine aminotransferase (ALT), aspartate aminotransferase (AST), and reactive protein C (CRP) using standardized kinetic method IFCC (International Federation for Clinical Chemistry) with pyridoxal phosphate.

#### Statistical analyses

Statistical analyze was performed using SPSS 16.0 for window software. Parameters values were expressed in mean value and  $\pm$ SD. P value lower than 0.05 was a criterion for statistical significance. The relationship between the different parameters analyzed was studied using Pearson correlations. To define and interpret association between variables we used adjusted regression models.

### Ethic consideration

All subjects were informed about the study protocol and all of them signed the informed consent before enrolling in the study. The protocol was approved by the Committee of Ethics of Clinical Emergency Hospital Bucharest.

### RESULTS

We report 126 patients being diagnosed as lead intoxication between the study period in our Toxicology Unit. Only data for 118 of them were complete, but 7 were lost from the procedure during the investigation so the final group consisted of 111 patients.

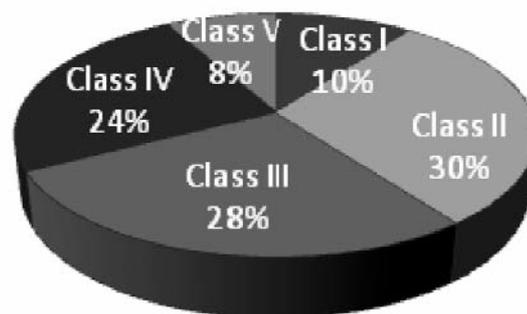
Baseline characteristics of the study population based on BLLs group are listed in **Table 1**. Age of the participants ranged from 22 to 58 years with a mean of  $44.5 \pm 4.7$ . Mean lead blood level was  $33.45 \mu\text{g/dl}$  (range 6.7 –  $73.6 \mu\text{g/dl}$ ). Considering age and weight of the participants, no differences were noticed according to blood lead levels groups.

Distribution of intoxication classes according to BLLs is demonstrated in **Fig. 1**. Most of our case had moderate lead intoxication.

**Table 2** shows alteration of selected biomarkers according to level of intoxication classified as BLLs classes.

ALT and AST variations were proportionally with the BLLs. There is no biochemistry test that can confirm liver disease, but ALT is a useful biomarkers before the lesions become irreversible and it is widely used. Removing the exposure, liver function can be reestablished.

The inflammatory marker, CRP plasma level, increased according to BLLs (class II-V had 2.56, 3.89, 4.56, 5.67 mg/L, respectively) with statistical differences between class III to V when compared to class I ( $p < 0.001$ ).



**Figure 1.** Distribution of subjects according to BLLs

When compared to the safest Class I, lower significant statistic values for SOD were found in Class III, IV and V, whereas the result was not significant for Class II.

### DISCUSSIONS

Evaluation of the present subjects indicated a higher incidence of lead exposure for the young adults coming mainly from rural areas. Unlike some other European similar reports, lead exposure in Romania is mainly due to illicit alcohol production in wide lead brass still. Anyway, the most severe forms are due to professional long term exposure.

Oxidative stress is described as the imbalance between two important processes: production of free radical species and detoxification the reactive intermediates [8]. Lead is capable of inducing both generation of reactive oxygen species and depleting the antioxidant reserves of the organism

Other studies have found the same data [9]. Medium class intoxication have a pro-oxidative status explained by

**Table 1. Characteristics of the study population**

	BLLs classes				
	Class I (N=11)	Class II (N=33)	Class III (N=31)	Class IV (N=27)	Class V (N=9)
Blood lead level, mean (rang), $\mu\text{g/dL}$	7.44 (4.23-9.64)				
Age, mean (SD), years	42.55 (3.15)	43.4 (2.76)	38.67 (4.4)	47.45 (3.8)	43.7 (4.6)
Weight, mean (SD) (kg)	85.7 (6.7)	84.7 (7.7)	88.7 (8.7)	92.7 (10.7)	84.6 (5.7)
Smoking habit (%)	44	34	64	23	46
Living area N (%)					
Rural	9 (81.81)	27 (81.81)	30 (96.77)	19 (70.37)	4 (44.44)
Urban	2 (18.18)	6 (18.18)	1 (3.22)	18 (29.62)	5 (55.55)
Education level N (%)					
low	3 (27.27)	25 (75.75)	17 (54.83)	16 (59.25)	4 (44.44)
medium	6 (53.91)	7 (21.21)	12 (38.70)	9 (33.33)	4 (44.44)
high	2 (18.81)	1 (3.03)	2 (6.46)	2 (7.42)	1 (11.12)
Illegally distilled alcohol (%)	44	53	57	14	11

Table 2. Alterations in biochemical markers in subjects with lead intoxication according to BLLs classes

	Class I (N=11)	Class II (N=33)	Class III (N=31)	Class IV (N=27)	Class V (N=9)
AST U/l (mean)	41.81 ± 14.43 (38.99–44.63)	42.33±12.33 <sup>NS</sup> (39.99–45.33)	41.81±14.43* (38.99–44.63)	41.81±14.43* (38.99–44.63)	41.81±14.43* (38.99–44.63)
ALT U/l (mean)	23.4 ± 1.3 (22.99–25.67)	22.33±12.33 <sup>NS</sup> (21.69–25.33)	32.71±8.23* (31.49–34.35)	31.81±14.43* (31.19–33.63)	41.81±12.43* (40.99–43.23)
CRP mg/L mean (SD)	2.45 (1.65)	2.56 (0.67) <sup>NS</sup>	3.89 (1.22)*	4.56 (1.05)*	5.67 (2.33)*
SOD U/g Hb mean, (SD)	1834.54(294.85)	2226.84(606.86) <sup>NS</sup>	2320.76(598.27)*	2461.59(664.46)*	2937.88(752.06)*
GP U/L mean (SD)	43.36 (13.29)	54.15 (19.01) <sup>NS</sup>	51.35 (16.95) <sup>NS</sup>	51.14 (17.83) <sup>NS</sup>	46.22 (11.22) <sup>NS</sup>

Values represent mean ± standard deviation and 95% CI for mean

\* significantly different from safest Class I

<sup>NS</sup> are not significant according to Tukey's test

higher SOD plasma values that balance the oxidative stress; for more severe long term intoxication SOD has lower values, indicating decreased levels of factors mediating the anti-oxidative barriers.

Inflammatory reaction developed by lead might be an explanation for other known effects upon humans, like elevated blood pressures, due some structural and functional changes in the endothelium [11]. Nevertheless, some of the previous studies reported both immune suppression and immune stimulating effects as consequences of lead exposure [12, 13].

### CONCLUSIONS

Although lead intoxication is ancient in the world it is still a persistent part of the public health actual concerns. The present data states the relationship between blood lead levels and oxidative stress markers at the cellular level. Monitoring lead exposure requires BLLs determinations as the most suitable methods whereas SOD values indicate the oxidative stress reactions magnitude.

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