ORIGINAL ARTICLE

BIOCHEMICAL PARAMETERS CHANGES INDUCED BY LEAD EXPOSURE

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Abstract

Lead exposure is still present in adult life, mainly due to work place circumstances, although sustained measures had been assessed to reduce environmental emissions. The aim of present study was to determine biochemical and haematological changes in individuals suffering from lead exposure in a particular population group. Patients in lead exposure group had significantly lower haemoglobin values than controls ($p = 0.015$), and higher values for gamma-glutamyl transferase and alkaline phosphatase ($p = 0.003$ and $p = 0.002$, respectively).

Rezumat

Scopul studiului a fost de a determina modificările biochimice şi hematologice, la persoanele expuse la plumb, într -un anumit grup de populaţie. Pacienţii din grupul expus la plumb au avut valori ale hemoglobinei semnificativ mai mici decât grupul control ($p = 0,015$), şi de asemenea valori mai mari pentru gama-glutamil transferaza şi fosfataza alcalină ($p = 0,003$, respectiv $p = 0,002$).

Keywords: lead exposure, biochemical parameters, gamma-glutamyl transferase, alkaline phosphatase

Introduction

Lead exposure is still present in adult life, mainly due to work place circumstances, although sustained measures had been assessed to reduce environmental emissions. One of the reference measures was performed back in the '80s, when introduction of lead free gasoline in the USA conducted to a decreased level of lead in air and in human blood, although previous gasoline was added with an organic form of lead known as tetraethyl lead [1]. Everyday life induces lead exposure, especially in some professional areas such as manufacturing or use of batteries, car radiators, pigments, cables and wires, ceramic ware with lead glazes, paint, cosmetics. Some other important sources of domestic lead exposure are represented by the use of paints and this is well known as an important intoxication source mainly for children [2]. Ayurvedic medication is also seen as lead exposure in some parts of the world [3], as well as various supplements used by women as slimming pills in order to lose weight [4]. Boxes used for dental intraoral imagistic film storage were analysed regarding potential lead exposure and a study performed by the Wisconsin Division of Public Health showed that those boxes contained a white powder found to be lead oxide [5]. In our country, several areas of non-ferrous metallurgical industry were found to exceed maximum levels for lead and cadmiu concentration measured in vegetables grown in those regions, as one study from Copşa Mică and Zlatna shows [6]. Also, some Danube area industrial towns from Romania, such as Galaţi, were found on risk in terms of high level of lead and other heavy metals in fish and water composition when compared with to samples from Tulcea [7].

The main activities associated with respiratory exposure to lead are scraping/ sanding/ burning leaded paint from surface. After entering the respiratory tract, inorganic lead is absorbed from the lungs, having a high absorption rate in adults, evaluated around 40%. On the contrary, gastro-intestinal absorption has an efficacy around 10 to 15% and this is increased in restricted diets, mainly in iron, phosphorus, calcium or zinc. On the other hand, children usually are intoxicated with lead through the gastrointestinal absorption, with a high efficacy (e.g. 50%) [8, 9]. After absorption, lead is conducted in various systems of the organism including soft tissues, skeleton and blood. The bio-cycle of lead in the bloodstream is well known: 99% binds the erythrocytes and free lead is present

in organs like kidney, brain or bone marrow. Its mean half-life is about 30 days considering normal renal function; still, in the skeleton, the half-life is about decades, and here is found around 95% of the body burden of lead [10]. Thus, lead biochemical clearance might be slower considering long term exposure individuals who store large amounts in their skeleton [11]. Some medical conditions such as pregnancy, breastfeeding, menopause or hyper-thyroidism might induce the release of some amount of the lead from the reservoir [12, 13, 14].

Illegally produced distilled alcohol represents another important source of lead exposure in some ethnic communities. In our country, there is a wide phenomenon of alcohol in-house production, especially in rural areas, using traditional methods. It is used for both private consumption and for sale. The natural source for the alcohol is represented by fermented fruits in large barrels for 6 to 8 weeks. In order to respect tradition, wood or charcoal represent the fire sources. Some of the final beverages can be contaminated due to materials used in the construction of the still. Particularly dangerous are considered stills involving parts of automotive radiators containing lead.

Biochemical considerations

Lead is an electropositive metal with a high affinity for negatively charged sulfhydryl groups; this is the mechanism through which lead induces the inhibition of some sulfhydryl dependent enzymes e.g. delta-aminolevulnic acid, dehydratase and ferrochelatase, known to be implicated in heme synthesis [1]. This is how plasma has a high level of measurable protoporphyrins due to disruption of haemoglobin synthesis. It is well known the anaemia in individuals with high levels of lead. In the same time, lead induces degradation of ribosomal RNA in red cells because of the inhibitory effect upon pyrimidine 5' nucleotidase, and this is seen as basophilic stippling on peripheral smear [15].

Brain damage induced by lead involves a permeability mechanism for blood brain barrier permitting accumulation in the astroglia [16].

One of the most important chemical mechanisms related to lead poisoning or environmental exposure is oxidative stress promoted by generation of hydrogen peroxide and superoxide radicals in human endothelial cells and vascular smooth muscle; this can furthermore be the explanation for high blood pressure effects [17].

Some recent data claim that global DNA methylation might be affected by lead, acting on deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) *via* mechanisms still to be discovered [18, 19].

The decline in cognitive function was associated with long-term lead burden and this was worse among hemochromatosis variant allele carriers than among wild-types; cognitive impairment was more important in individuals with a greater number of either variant alleles (H63D or C282Y) [20].

The aim of present study was to determine the biochemical and haematological changes in individuals suffering from asymptomatic lead exposure due to illicit alcohol consumption, when compared to a control group.

Materials and Methods

A total of 73 patients were enrolled as study group consisting in patients admitted for lead exposure in Critical Care Toxicology Unit of Bucharest Clinical Emergency Hospital between 1.02.2014 - 1.02.2015. Out of the initial group of 73 patients, 13 discontinued the study due to incomplete data, while another 8 patients didn't consent to end the follow-up procedure. The remaining 52 patients represents the final study group. We picked a control group consisting in 16 healthy individuals, presented to our Toxicology laboratory for various blood determinations, used for comparison. The study protocol was approved by the Hospital Committee of Ethics and all participants gave their written consent before enrolling the study.

Social demographic characteristics

Each patient had a personal file including data about: age, sex, environmental (urban/rural), illegally distilled alcohol production (yes/no), education level (subjects who graduated from college were considered to have achieved a high level of education; those having a high school or grade school diploma were considered to have achieved medium level, while those having completed at least 4 grades were considered to have a low educational level) and smoking habits (yes/no).

All participants underwent a test for alcohol consumption. We used The Alcohol Use Disorders Identification Test (AUDIT) validated to detect alcohol issues experienced in the last year. A score of 8+ on the AUDIT generally indicates harmful or hazardous drinking [21].

Sampling and laboratory data

Blood was collected by venepuncture after informed consent was obtained for all patients; we used plain tubes filled with 10 mL of blood in order to obtain serum and another 15 mL in anti-coagulant tubes using EDTA-Na_2 (ethylene-diaminetetraacetic disodium acid solution). Serum levels of various parameters were noted at the admission visit: haemoglobin (Hb), mean corpuscular volume (MCV), aspartate aminotransferase activity (AST), alanine aminotransferase activity (ALT), gamma-glutamyl transferase activity (γGT), alkaline phosphatase activity (ALP) and serum iron (Fe). Patients suffering from chronic illness that could affect the study parameters were excluded from the study, initially. With time, liver disease passes

through several stages including hepatitis, liver cirrhosis and liver cancer. Heavy metals induce organ structural damaging and liver transaminases are often used to monitor the severity of lesions. γGT is an enzyme found in liver and kidneys but the plasma level mainly originates from liver through a cellular injury mechanism [22].

In the meantime, lead levels were recorded for every patient in both groups, intoxication being considered for lead serum levels higher than 40 μg/dL, using atomic absorption spectrophotometry provided by Spectra AA-880.

All patients were investigated using ultra-sonography in order to assess liver parenchyma and detect possible liver lesions. Also, after inclusion in the study, subjects' general practitioners were addressed to reveal recent medical records and investigations of the patients included in the study.

Blood pressure

In order to assess blood pressure (BP), authors used Nihon Kohden MU-671 RK monitor, measured twice, in seated position and the average of two readings was recorded. Subjects using antihypertensive medication were excluded from the study.

Subjects were considered normotensive if diastolic BP ≤ 90 mmHg and systolic BP < 140 mmHg and hypertensive if their diastolic BP > 90 mmHg and systolic BP ≥ 140 mmHg.

Statistical analyses

Mann-Whitney U test was used in order to perform comparison between data provided by the enrolled patients. Statistical significance was considered for p values lower than 0.05. The statistical analysis was performed using SPSS 13.0 software.

Results and Discussion

Social demographic characteristics

Mean age was similar for both groups, 36.4 ± 6.73 years *vs.* 38.7 ± 7.33 years. Most of the patients belong to rural areas as seen in Table I.

Table I

General characteristics of the selected study population

	Lead group				Control group			
	Sample	Mean or %	95% CI from	to	Sample	Mean or %	95% CI from	to
Mean age (years)		36.4 ± 6.73				38.7 ± 7.33		
Sex (%)								
male	34	65.38	49.39	81.37	10	62.5	32.49	92.51
female	18	34.61	12.63	56.59	6	37.5	-1.47	75.87
Living area								
urban	11	21.15	-2.98	45.28	5	31.25	-9.38	71.88
rural	41	78.84	66.34	91.34	11	68.75	41.36	96.14
Illegally distilled alcohol								
yes	42	80.76	68.84	92.68	2	12.5	-33.33	58.33
no	10	19.23	-5.2	43.66	14	87.5	70.18	104.82
Education level								
low	39	75	61.41	88.59	8	50	15.35	84.65
medium	11	21.15	-2.98	45.28	5	31.25	-9.38	71.88
high	2	3.84	-22.79	30.47	3	18.75	-25.42	62.92
Smoking								
yes	32	61.53	44.67	78.39	7	43.75	7	80.5
no	20	38.46	17.14	59.78	9	56.25	23.84	88.66

As seen in Table I, 80% of participants are traditional alcohol producers, and the remaining 19.23% declared they buy such Romanian beverages from their neighbours or from other rural communities.

AUDIT questionnaire revealed scores under 8 for both groups. Medical history and previous bio-chemical investigations were within normal ranges, as shown through the general practitioners registry.

Haematological and biochemical data

As we were expected, anaemia is a well-known characteristics of lead intoxicated patients, so lead intoxication group patients had significantly lower Hb values than controls (p = 0.015) (Table II). As a marker for anaemia, Hb values and MCV were found to be proportionally decreased with lead plasma levels, as seen in some previous studies. Pala *et al.* also found lower mean values for Hb and MCV [23].

AST and ALT mean values were 23.5 ± 2.2 U/L and 25.6 ± 3.3U/L, respectively in the lead group while 24 ± 4.2U/L and 27 ± 4.5U/L were the data available for the control group. Chang *et al.* published large data upon professional exposure to lead and organic solvents and his results also showed that lead exposure did not influence the hepatic laboratory tests [24]. In our study, no statistical significance could be established in

relationship with liver enzymes. In order to assess liver chronic damage, extended tests should be performed. The evaluation of γGT and ALP showed that out of the biochemical parameters, those were the only ones correlated with lead intoxication ($p = 0.003$ and $p = 0.002$, respectively). Both mean values were higher in the lead exposed subjects than in the control ones (Table II). Ultrasonography showed normal liver structure except for 6 individuals in the Lead group and 4 in the Control group, diagnosed with mild or moderate hepatic steatosis. Doppler ultrasound examination revealed normal hepatic blood flow in all cases.

Laboratory findings regarding iron metabolism in lead exposed subjects are inconstant. Our data showed that individuals having high blood lead values were more likely to have decreased values of iron when compared to control group. Some of the recent published research showed that there is an iron transport channel in the intestine called divalent metal transporter (DMT); this is regulated by the iron regulatory proteins 1 and 2 [25] and it serves not only for the iron transport, but also zinc, lead and copper. Anyway, the iron metabolism investigation in lead exposed patients highly depends upon either respiratory or digestive mechanisms of intoxication.

Table II

Haematological and biochemical data for the studied groups

Parameters evaluated	Lead group (n = 52) Mean ± SD	Control group (n = 16) Mean ± SD	p
Pb (microg/dL)	49 ± 11.4	4.25 ± 1.2	0.001
Hb (g/dL)	11.7 ± 1.6	14.4 ± 2.1	0.015
MCV (f/L)	82.9 ± 4.9	91.2 ± 5.5	0.001
GGT (mg/dL)	31.4 ± 4.5	26.8 ± 9.4	0.003
ALP (UI/L)	88 ± 15.3	58 ± 19.8	0.002
ALT (U/L)	25.6 ± 3.3	27 ± 4.5	NS
AST (U/L)	23.5 ± 2.2	24 ± 4.2	NS
Fe (μ/dL)	71 ± 35	89 ± 36	0.002

Blood pressure

Table III shows how high blood pressure is related to blood lead level, as it has been described before in the literature [26, 27]. 32.6% of total subjects were having hypertension and this was related to high lead blood concentrations. Some of these concluded in a weak association between lead level exposure and the risk for hypertension [28], but on the contrary, Scinicariello *et al.* and Den Hond *et al.* stated that this association exists [29, 30]. This might be also due to the differences in target population, study design, evaluated parameters and type of associations.

Table III

Blood pressure status and mean blood lead level of the subjects having high lead levels

Blood pressure	Frequency (%)	Mean blood lead (μg/dL)	SD	p
Normal	35 (67.30)	59.08	17.03	
High	17 (32.69)	77.47	15.29	
Total	52	65.09	18.51	0.016

Conclusions

We identified potential risk factors for lead exposure in a particular group of Romanian rural population using in-house alcoholic beverages. Considering the important effects upon haematology and biochemical parameters of lead exposure, special attention should be carried out in several parts of each country where artisanal alcohol manufacturing is a habit. Increasing awareness among control institutions of these health issues might lower the level of lead exposure in vulnerable populations.

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