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Relationship between lead absorption and iron status and its association with oxidative stress markers in lead-exposed workers

Michał Słota [a], Marta Wąsik [b], Tomasz Stołtny [c], Anna Machoń-Grecka [d], Aleksandra Kasperczyk [d], Francesco Bellanti [e], Michał Dobrakowski [d], Artur Chwalba [f], Sławomir Kasperczyk [d,*]

[a] ARKOP Sp. z o.o., Kolejowa 34a, 32-332, Bukowno, Poland
[b] Department of Clinical Biochemistry and Laboratory Diagnostics, Institute of Medicine, University of Opole, Oleska 48, 45-052, Opole, Poland
[c] District Hospital of Orthopaedics and Trauma Surgery in Piekary Śląskie, Bytomska 62, 41-940, Piekary Śląskie, Poland
[d] Department of Biochemistry, Faculty of Medical Sciences in Zabrze, Medical University of Silesia in Katowice, Poniatowskiego 15, 40-055, Katowice, Poland
[e] Department of Medical and Surgical Sciences, University of Foggia, Viale Pinto 1, 71122, Foggia, Italy
[f] Department of Pharmacology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia in Katowice, Poniatowskiego 15, 40-055, Katowice, Poland

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ABSTRACT

Background: The emission of lead (Pb) occurring during the extraction, processing and industrial applications of this element remains a significant environmental risk factor. The absorbability of lead in humans is strongly associated with the general health status of exposed individuals. Existing mineral deficiencies are considered being a predisposition to an increased Pb uptake. Both, iron deficiency and lead poisoning are the major causative factors responsible for the prevalence of anemia within the vulnerable population, especially in children. Although some of the intervention programs of counteracting lead poisoning by iron supplementation proved to be effective in the Pb-exposed population, the exact mechanisms of this interaction still require further studies. The objective of the presented study was to examine the association of iron level on oxidative stress measures and its effects on the severity of lead toxicity in the exposed population.

Methods: The analyzed population consisted of 270 male workers from the lead-zinc smelter. The studied population was divided into two sub-groups based on the serum iron concentration: low iron level group (L-Fe; Fe < median value) and high iron level group (H-Fe; Fe > median value). Measured traits comprised of blood lead (PbB), serum Fe and zinc protoporphyrin (ZPP) levels as well as a blood count and oxidative stress markers.

Results: No significant correlation between serum iron concentration and PbB in the tested cohort was found. On the contrary, the analysis of ZPP levels (long-term marker related to a hematologic toxic effect of Pb) within the subgroups differing in serum Fe level shown that ZPP was 12.3 % lower ($p = 0.043$) in subjects classified within the H-Fe group. A positive correlation of serum Fe and total antioxidant capacity (TAC) was found ($R = 0.1999$). The conducted 3-D PCA analysis showed that individuals classified within the H-Fe group were characterized by the co-occurrence of higher Fe levels, lower ZPP, and higher TAC value.

Conclusion: These results support the existing evidence providing that maintaining the optimal status of Fe may play a significant role in preventing the lead poisoning and alleviating harmful effects of Pb on the oxidative balance in humans.

1. Introduction

Lead (Pb) is a chemical element belonging to the class of heavy metals that are environmental pollutants posing a considerable health risk for the human population. Due to the chemical properties of lead - low melting point, high malleability, and corrosion resistance – it is still widely applied in different sectors of industry. Major emission of lead is associated with vehicle traffic (aviation gas residual Pb in car fuel after the introduction of unleaded petrol, engine lubricants, tire and brake wear), iron and steel production processes, non-ferrous metal casting, energetics, and chemical industry. The accumulation of lead traces result in contamination of the biosphere and thus exposure to living

* Corresponding author.
E-mail address: skasperczyk@sum.edu.pl (S. Kasperczyk).

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organisms. Although since the year 1990, emissions of lead in EEA-33 countries decreased by 93 % [1], it remains a health risk factor for exposed workers and vulnerable populations.

The occupational exposure to lead is mainly associated with the inhalation of fumes and skin contact. The routes of Pb-exposure to the general population are comprised of oral ingestion with food and water, intoxication via. cosmetics and medicines, as well as contaminated consumer goods [2]. The absorbability of lead depends on its physico-chemical properties (i.a. particle size, chemical form, and solubility) as well as the general health status of the exposed individual. It has been shown that the factors related to the human diet, with a special emphasis to pre-existing nutritional deficiencies, can significantly affect the uptake of toxic elements. Higher lead absorption has been observed in case of insufficient supply with calcium, zinc, and iron [3]. Since lead can displace some physiological metal cofactors from active sites and disrupt the natural enzymatic processes, the spectrum of toxicity to biological macromolecules and interference of subcellular structures stability is considerably wide. One of the most classical symptoms that are commonly observed after a chronic exposure to lead is a Pb-toxicity-related anemia [4]. The reduction in heme levels is mostly attributed to the interference of lead with a heme biosynthesis enzyme – delta-aminolevulinic acid dehydratase (ALAD). There is a strong synergistic effect of lead poisoning and iron deficiency that both contribute to the occurrence of anemia [5]. Epidemiological studies proved that the correlation of Fe depletion and alleviated Pb concentrations in blood and incidence of anemia is predominant in a group of young children between 1–2 years [6].

Besides the direct effect on enzymatic activities, a high concentration of heavy metals may cause secondary oxidative stress resulting from the generation of ROS (reactive oxygen species) and inhibition of antioxidant enzymes [6]. It has been demonstrated that lead inhibits the activity of certain enzymes, such as superoxide dismutase and glutathione peroxidase, that are involved in free radicals scavenging in the organism [7]. Recent studies have also shown that Pb-dependent ROS formation can negatively affect the iron status, by disrupting Fe loading to ferritin [8].

2. Material and methods

2.1. Study population

Approval for the following protocol of the study was granted by the Bioethics Committee of the Medical University of Silesia (Permission No. KNW/022/KB1/108/14).

The examined population comprised of 270 male subjects working under occupational exposure to lead in a lead-zinc smelter in Miasteczko Śląskie, Poland. Conducted monitoring of health surveillance involved the collection of epidemiological data (i.a. age, duration of employment in Pb exposure, smoking habits, and medical records) as well as overall health status assessment. Body mass and height were measured in order to calculate body mass index (BMI). The analyzed markers of lead exposure involved the measurements of the concentration of Pb in whole blood (PbB) and zinc protoporphyrin (ZPP). Eligibility criteria for the conducted study were based on the presence of occupational Pb exposure and reported level of PbB exceeding 20 μg/dl. Subjects with a history of chronic diseases and/or any symptoms of lasting infectious diseases were excluded from the study.

The study population was divided into two sub-groups based on the median serum iron concentration (20.62 μmol/l): low iron level group (L-Fe group, n = 135) and high iron level group (H-Fe group, n = 135). Serum iron levels ranged from 7.29 to 20.59 μmol/l in the L-Fe group and 20.64–61.36 in the H-Fe group respectively.

2.2. Samples collection

Blood samples from each participant of the study were collected by venipuncture from the basilic vein of the forearm, using sterile test tubes coated with K_3EDTA (Vacuette; Greiner-Bio, Frickenhausen, Germany) as an anticoagulant to obtain 15 ml of whole blood, whereas a blood sample volume of 10 ml was collected into plain tubes to obtain serum. Using the whole blood, PbB and ZPP were determined. The remaining blood was centrifuged (3,000 rpm for 10' at 4 °C) to obtain the sediment of erythrocytes that was subsequently rinsed three times using 0.9 % NaCl. Following that erythrocytes were hemolyzed with deionized water and the activity of studied oxidative stress markers, antioxidant enzymes concentration, and hemoglobin levels were evaluated. Additional markers of oxidative stress and antioxidant enzymes concentrations were measured in obtained blood plasma. Blood serum was collected by centrifugation and used for the analytical measurement of iron concentrations.

2.3. Determination of blood lead

The analysis of the blood lead level (PbB) was conducted by graphite furnace atomic absorption spectrometry technique using the iCE 3400 AAS Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The device was calibrated with the CliniCal® standard - Whole Blood Calibration (lyophilized whole blood) - Recipe Chemicals + Instruments (Germany). Using a reference material, the precision within one and several series of measurements was determined (repeatability index cV = 0.8 %). Additional validation of Pb measurements was also carried by periodic external control materials originating from the Centers for Disease Control and Prevention, Atlanta (USA). The principle of the method is based on diluting the blood with the modifier mixture containing $NH_4H_2PO_4$, Triton X-100, and HNO_3. The determination of lead concentration in the blood solution was carried using the flameless atomic absorption spectrometry technique by measuring the absorbance at 283.3 nm (alternative wavelength − 217 nm). The obtained results were expressed as [μg/dl].

2.4. Determination of zinc protoporphyrin (ZPP)

The blood concentration of zinc protoporphyrin was measured directly using Aviv Hematofluorometer HF Model 206 (Aviv Biomedical, Lakewood, NJ, USA) using an excitation wavelength of 415 nm and an emission wavelength of 596 nm. The radiation from the halogen lamp is monochromatized (415 nm) by an intrusion filter and directed to a drop of blood placed on a coverslip. The excited fluorescence radiation passes through an interference filter of a 596 nm wavelength that is characteristic of ZPP and reaches the photomultiplier tube. The instrument measures the ratio of the fluorescent substance (ZPP) to the concentration of the substance that absorbs light in the reference sample (hemoglobin alone). For the purpose of measurement validation, Aviv ZPP Red Blood Cell Controls (Biomedical Inc., USA) test blood solutions were used. The results were expressed as μg ZPP per g of hemoglobin ([μg/g] Hb).

2.5. Determination of iron concentration

The determination of the level of iron in the serum was conducted using the colorimetric method with the application of the FerroZine method, without deproteinization of the sample. The measurements were performed using the PZ Cormay reagent kit (cat. no.: 7-458) together with the Multikalibrator kit K6504-03 (Alpha Diagnostics). Automated biochemistry analyzer - BS-200E (Mindray, Shenzhen, China) was used in the assay. In the assay, iron ions present in the sample react with ferrozine to form a colored complex. Quantification of the color reaction based on the calibration solutions allows determining the relative level of iron in the tested sample.

2.6. Blood count

The assessment of complete blood count parameters was conducted using analyzer Sysmex K-4500 Automated Hematology Analyzer (Sysmex Corporation, Kobe, Japan). Following measurements were obtained: white blood cells (WBC) count, red blood cells (RBC) count, hemoglobin (HGB) level, hematocrit (HCT), platelet (PLT) count.

2.7. Markers of oxidative stress

The parameter of total antioxidant capacity (TAC) was measured in blood plasma according to the adopted protocol of Erel [9]. A colored 2, 2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid radical cation (ABTS*+) solution is decolorized by the antioxidants present in the analyzed sample. The reaction efficiency depends on antioxidants concentrations. The color change was measured as a change in absorbance at 660 nm using an automated analyzer. Obtained results were expressed as [mmol/l].

Total oxidant status (TOS) was measured in blood plasma according to compatible methodology [10]. The measurement protocol is based on the oxidation of ferrous ions to ferric ions in the presence of various oxidant species in an acidic medium. The measurement of the ferric ion by xylenol orange is obtained using an automated analyzer. Obtained results were expressed as [pmol/l]. Oxidative stress index (OSI) was calculated as the percentage ratio of TOS to TAC.

The concentration of bilirubin in blood plasma was measured using Stat-Analyzer (PerkinElmer, Waltham, MA, USA) according to the manufacturer's instructions. A used analyzer determines total bilirubin concentration by spectrophotometric analysis. Bilirubin concentrations were expressed as [pmol/l]. A rationale behind considering bilirubin an oxidative stress-related marker was based on earlier evidence proving that the administration of bilirubin was associated with elevated glutathione (GSH) level and increased activity of antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [45].

The methodology of Arab and Steghens [11] was adopted to measure the concentrations of lipid hydroperoxides (LPH) in blood plasma. In this assay oxidation of Fe II to Fe III by lipid hydroperoxides, under acidic conditions. The complexation of Fe III by xylenol orange is quantitatively detected by using an automated analyzer. The measurements were expressed in [pmol/l].

The concentration of malondialdehyde (MDA) - a lipid peroxidation marker, was measured fluorometrically as a 2- thiobarbituric acid-reactive substances (TBARS) in plasma according to the standard methodology of Ohkawa, Ohishi and Yagi [12] with introduced modifications. Tested samples were mixed with 8.1 % sodium dodecyl sulfate, 20 % acetic acid, and 0.8 % 2-thiobarbituric acid. After vortexing, samples were incubated for 1 h at 95 °C and butanol-pyridine 15:1 (v/v) solution was added. The mixture was shaken for 10 min and then centrifuged. The butanol-pyridine layer was measured fluorometrically at 515 nm and 522 nm excitation wavelengths (PerkinElmer, Waltham, MA, USA). The levels of 2- thiobarbituric acid-reactive substances were expressed as MDA equivalents. Tetraethoxypropane was used as the standard. Concentrations were expressed in [pmol/l] (plasma) and [nmol/g] Hb (erythrocytes).

The concentrations of lipofuscin (LPF) were tested according to the methodology of Tsuchida et al. [13]. Lipofuscin has a specific fluorescence characteristic with excitation and emission maxima at 355–360 and 430−435 nm for human-origin samples. Measurements were expressed in [RU/l] (relative unit, RU) and [RU/g] Hb (erythrocytes).

The concentrations of protein sulfhydryl groups (PSH) were evaluated in accordance with the method by Koster, Biemond, and Swaak [14]. The rate of reaction of DTNB, undergoing reduction of protein-bound compounds containing thiol groups, was assessed by the yield of the yellow anion derivative, 5-thio-2-nitrobenzoate, which absorbs at a wavelength of 412 nm with the use of PerkinElmer automated analyzer (PerkinElmer, Waltham, MA, USA). The results were expressed as [pmol/g] in protein and [pmol/l] in plasma.

The level of ceruloplasmin (CER) concentration in blood plasma was analyzed in accordance with the protocol described by Richterich [15]. The results were expressed in [mg/dl].

2.8. Antioxidant enzymes

For the assessment of the activity of superoxide dismutase (SOD) in blood plasma and erythrocytes, the methodology of Oyanagui [16] was applied. Activities of superoxide SOD enzyme were expressed in nitric units (NU) in each ml as [NU/mL] (plasma) and [NU/mg] Hb (erythrocytes). The activity of catalase (CAT) in erythrocytes was evaluated following the method of Johansson and Håkan Borg [17]. Measurements were conducted using a PerkinElmer automated analyzer (PerkinElmer, Waltham, MA, USA). The activity of CAT was expressed as klU/g Hb. The activity of glutathione reductase (GR) in erythrocytes was tested according to Richterich [15] using a PerkinElmer automated analyzer. The activity measurements were expressed as pmoles of NADPH utilized per minute per g of hemoglobin in erythrocytes ([lU/g] Hb). The activity of glutathione S-transferase (GST) in erythrocytes was evaluated by the kinetic method described by Habig and Jakoby [18]. The activities of GST were tested using an automated PerkinElmer analyzer. Obtained results were expressed as pmoles of thioether produced per minute per g of hemoglobin in erythrocytes ([mlU/g] Hb). The activity of glutathione peroxidase (GPx) in erythrocytes was measured by the kinetic method of Paglia and Valentine [19]. The activity of the GPx enzyme was expressed as μmoles of NADPH oxidized per minute per g of hemoglobin in erythrocytes ([lU/g] Hb).

2.9. Statistical analysis

Statistical analysis was performed using Statistica 12.0 PL software (StatSoft Polska). Descriptive statistics were reported as mean ± standard deviation (SD) for normal distribution. Initial Shapiro-Wilk's test was used to determine the normality of distribution of each tested variable, and Levene's test was applied to verify homogeneity of variances. Statistical comparisons were carried with an application of the student's t-test, Mann- Whitney U test, and chi-squared test. Spearman's rank-order correlations coefficients were calculated for the assessment of existing associations between tested variables. PCA (Principal Component Analysis) was performed for the recognition of existing associations of more than two components. A probability at $p \leq 0.05$ was considered statistically significant.

3. Results

3.1. Health status & epidemiological data

No statistically significant differences in age, duration of employment, weight, height, and smoking habits between individuals classified

Table 1
Health surveillance and epidemiological data collected from tested population.

Variable		L-Fe Mean ± SD	H-Fe Mean ± SD	*p*
Age [years]		42.7 ± 9.47	42.7 ± 8.94	0.995
Seniority [years]		16.4 ± 8.69	17.1 ± 8.58	0.495
Height [cm]		176.6 ± 6.29	176.9 ± 5.69	0.743
Weight [cm]		86.3 ± 13.68	88 ± 12	0.406
DISEASES [%]	Diabetes	0	8	0.015
	Coronary diseases	3	1	0,532
	Hypertension	18	14	0,559
Active smoking [%]		40	30	0.185

Normality testing as per Shapiro-Wilk's test; p value (t-test), $p < 0.05$; Subgroups: L-Fe (Fe level below median) and H-Fe (Fe value above median).

among L-Fe and H-Fe subgroups were observed (Table 1). The individuals classified in both subgroups did not differ in terms of the occurrence of chronic diseases, such as coronary diseases and hypertension. On the contrary, the increased frequency of the individuals diagnosed with diabetes has been noted in the H-Fe group (8 % of tested subjects, compared to no cases in the L-Fe group). The measured mean Fe level in diabetics within the H-Fe group was >30 μmol/l.

3.2. Association of body iron status and lead exposure markers

The analysis of lead exposure markers in the groups differing in statutory blood iron levels, shown that in a high Fe subgroup, mean PbB was 4.7 % higher ($p = 0.005$). On the contrary mean ZPP level was 12.3 % lower ($p = 0.043$) in subjects within the H-Fe group when compared to the L-Fe subgroup (Table 2).

The differences in blood count parameters in the subjects classified within the subgroups were not statistically significant except the hemoglobin level that was 2 % higher in the H-Fe group when compared to the L-Fe group ($p = 0.014$) (Table 3).

The analysis of the markers of oxidative stress exposure tested in blood plasma shown a significant difference in total antioxidant capacity (TAC), oxidative stress index (OSI), and bilirubin level (Table 4). Subjects from L-Fe were characterized by a higher mean OSI index by 20.86 %, as well as lower TAC by 3.57 % compared to the H-Fe group. The level of bilirubin was 15 % higher in subjects classified within H-Fe groups when compared to the L-Fe group.

The assessment of oxidative stress markers in erythrocytes in subjects within L-Fe and H-Fe groups demonstrated a significant difference in catalase (CAT) level that was ~4% higher in the group characterized by an iron level below the median value. Other minor differences in the rest of the tested markers were no statistically significant between both subgroups (Table 5).

The summary of the existing positive and negative correlations ($p < 0.05$) between serum iron level, lead exposure markers, blood count parameters, and oxidative stress markers in the tested cohort is presented in Table 6.

Performed PCA (Principal Component Analysis) computations were undertaken to better understand the joint effects of iron status, lead exposure markers, and antioxidative status indices. Preliminary analyses resulted in the selection of the following principal components (PCs) based on correlation matrix: Pc1 – blood serum Fe [μmol/l]; Pc2 - ZPP mean concentration; Pc3 – total antioxidative capacity (TAC) [mmol/l]. Selected principal components were characterized by the highest eigenvalue, or the percentage of explained variance of the measured subset of variables. The evaluated total variance explained (TVE) was 64.31 %, with the following share of individual components: Pc1 = 28,01 %, Pc2 = 19,45 % and Pc3 = 16,85 %. Conducted 3D-PCA analysis proved the distinctiveness of pre-defined subgroups that were classified based on the Fe level above or below the median value. Analysis of the tested variables over the 3D matrix demonstrated a clear differentiation of L-Fe subgroup (red dots on the plot), that was characterized by a lower Fe level (as per the study design), higher ZPP concentration, and lower TAC, whereas H-Fe group (green dots on the plot) was defined by a higher Fe level, lower ZPP and higher TAC value (Fig. 1).

Table 2
Iron blood serum level and lead exposure markers: concentration of Pb in whole blood (PbB) and zinc protoporphyrin (ZPP) among selected subgroups.

Variable	L-Fe Mean ± SD	H-Fe Mean ± SD	*p*
Iron (Fe) [μmol/l]	16.78 ± 2.73	27.20 ± 6.80	<0.001
Lead (Pb) [μgl/dl]	36.43 ± 4.78	38.14 ± 5.04	0.005
ZPP [μg/g Hb]	6.19 ± 3.23	5.43 ± 2.39	0.043

Normality testing as per Shapiro-Wilk's test; p value (U Mann-Whitney test), $p < 0.05$; L-Fe (Fe level below median) and H-Fe (Fe value above median).

Table 3
Blood count parameters of subjects among selected subgroups.

Variable	L-Fe Mean ± SD	H-Fe Mean ± SD	*p*
WBC [G/l]	7.24 ± 1.71	7.13 ± 2.06	0.432
RBC [T/l]	4.84 ± 0.34	4.87 ± 2.36	0.386
HGB [g/dl]	15.02 ± 0.84	15.32 ± 0.89	0.014
HTC [%]	42.76 ± 2.17	43.24 ± 2.51	0.140
PLT [G/l]	238.1 ± 47.7	231.6 ± 57.4	0.266

Normality testing as per Shapiro-Wilk's test; p value (U Mann-Whitney test), $p < 0.05$; WBC - white blood cells count; RBC - red blood cells count; HGB - hemoglobin, HCT - hematocrit; PLT - platelets. L-Fe (Fe level below median) and H-Fe (Fe value above median).

Table 4
Oxidative stress markers tested in blood plasma among selected subgroups.

Variable	L-Fe Mean ± SD	H-Fe Mean ± SD	*p*
TOS (μmol/l)	11.31 ± 5.47	10.25 ± 4.16	0.113
LPH (μmol/l)	3.27 ± 2.07	3.16 ± 2.05	0.675
MDA (μmol/l)	2.89 ± 1.19	3.01 ± 1.21	0.243
LPF (RU/l)	584.5 ± 100.6	563.1 ± 97.3	0.137
OSI (%)	1.15 ± 1.22	0.91 ± 0.39	0.041
TAC (mmol/l)	1.12 ± 0.12	1.16 ± 0.11	0.005
Bilirubin (μmol/l)	0.68 ± 0.47	0.80 ± 0.41	0.012
PSH (μmol/g protein)	4.16 ± 0.54	4.24 ± 0.54	0.368
CER (mg/dl)	42.36 ± 5.87	43.7 ± 6.03	0.091
SOD (NU/mL)	20.37 ± 3.0	19.84 ± 3.97	0.197

Normality testing as per Shapiro-Wilk's test; p value (U Mann-Whitney test), $p < 0.05$; TOS - total oxidant status; LPH - lipid hydroperoxides; MDA - malondialdehyde; LPF - lipofuscin; OSI - oxidative stress index; TAC total antioxidant capacity; PSH - protein sulfhydryl groups; CER - ceruloplasmin; SOD - superoxide dismutase. L-Fe (Fe level below median) and H-Fe (Fe value above median).

Table 5
Oxidative stress markers tested in erythrocytes among selected subgroups.

Variable	L-Fe Mean SD	H-Fe Mean SD	*p*
MDA (nmol/g Hb)	229.8 ± 65.3	222.2 ± 69.5	0.230
LPF (RU/g Hb)	644.5 ± 201.7	653.1 ± 214.4	0.711
GR (IU/g Hb)	6.93 ± 1.41	6.88 ± 1.38	0.759
GST (mIU/g Hb)	185.6 ± 72.3	174.3 ± 67.0	0.091
GPX (IU/g Hb)	51.35 ± 13.14	50.82 ± 13.38	0.666
CAT (kIU/g Hb)	467.5 ± 66.2	449.0 ± 63.2	0.049
SOD (NU/mg Hb)	195.3 ± 32.9	191.2 ± 32.5	0.345

Normality testing as per Shapiro-Wilk's test; p value (U Mann-Whitney test), $p < 0.05$; MDA - malondialdehyde; LPF - lipofuscin; GR - glutathione reductase; GST - glutathione S-transferase; GPX - glutathione peroxidase; CAT - catalase; SOD - superoxide dismutase. L-Fe (Fe level below median) and H-Fe (Fe value above median).

Table 6
Summary of proved positive and negative correlations between serum iron level and other factors.

Correlation between Fe level and tested parameter	ρ	p
ZPP (μg/g Hb)	−0.2011	0.019
Bilirubin (mg/dl)	0.2022	0.039
TAC (mmol/l)	0.1999	0.001
CER (mg/dl)	0.1695	0.005
CAT (kIU/g Hb)	−0.1240	0.042

ρ - Spearman's rank-order correlations coefficient; $p < 0.05$; ZPP - zinc protoporphyrin; TAC - total antioxidant capacity; CER - ceruloplasmin CAT – catalase.

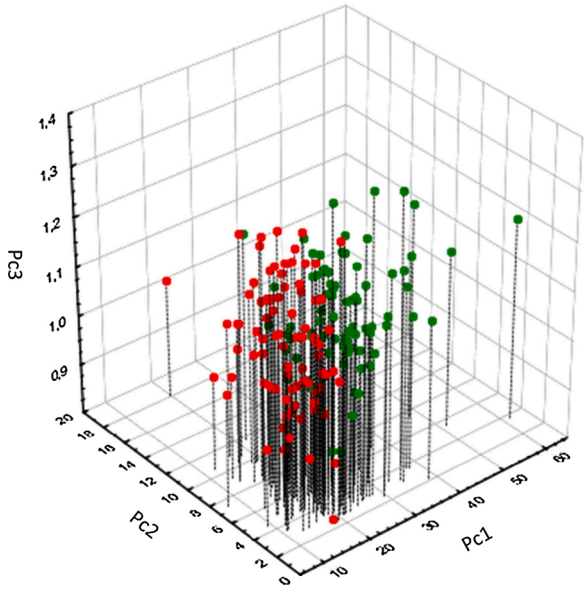

Fig. 1. A three-dimensional PCA plot (Pc1, Pc2, and Pc3) generated from principal component analysis (PCA) of the quantitative variables related to the iron status, lead exposure markers, and antioxidative status indices. The red dots indicate the data points corresponding to the L-Fe group (Fe level below median), whereas green dots correspond to H-Fe (Fe value above median) subgroup. *Pc1 – blood serum Fe [μmol/l]; Pc2 - ZPP level [μg/g Hb]; Pc3 - TAC* [mmol/*l*].

4. Discussion

4.1. Iron status related health surveillance

The analysis of epidemiological data collected from subjects involved in the carried study demonstrated an increased frequency of the individuals diagnosed with diabetes in the H-Fe group (8% of tested subjects), comparing to the lack of cases in the L-Fe group. Since iron is considered a strong pro-oxidant, the co-occurrence of an increased level of oxidative stress and high body iron status indices can be associated with a higher risk of type 2 diabetes [20]. Iron overload is associated with diabetes risk, having a direct and causal role in diabetes pathogenesis mediated both by β-cell failure and insulin resistance. The mechanism involved in this process includes the modulation of adipokines and Fe-dependent modification of intracellular signal transduction pathways [21]. On the contrary, impaired iron uptake could be an important factor that affects glucose metabolism [20]. Iron deficiency is also associated with obesity, which is another major risk factor for diabetes [21]. Both these markers - ferritin levels and BMI - are often considered as independent predictors in a glucose tolerance test [20].

4.2. Association of body iron status and blood lead level

Lead, iron, and some other divalent cations are involved in certain interactions and often compete in their uptake systems. Adequate iron status is considered to possess a dual function in alleviating lead toxicity. First of all, as most environmental lead is absorbed in the intestine, normative iron intake favors Fe competition with Pb against DMT1 transporter, to which Pb has a partial affinity. Secondly, sufficient iron body stores affect the expression of the *DMT1* gene, conditioning the number of available transporters within the duodenum [5]. Suboptimal dietary intake of Fe may thus result in increased absorption of lead, due to the existing antagonism in the absorption pathways of these metals. Lead has been also found to bind and interfere with enzymes, that are crucial for the uptake, transport and utilization of iron: transferrin, mucin, mobiferrin, hemoglobin, and certain enzymes of the heme synthesis pathway [5].

The conducted study has not proved a significant correlation between serum iron concentration and blood lead level in the tested cohort. The reason for this observation may be attributed to the specificity of the tested iron status parameter. Measurable Fe-status markers that are applicable in the human population screening comprise of transferrin saturation, ferritin level, hematocrit, hemoglobin level, iron/total iron-binding capacity (Fe/TIBC), red cell distribution width (RDW), and total serum Fe content [5]. Some of these markers are considered to be more sensitive to certain fluctuations of iron body stores, while others are less affected. Negative correlations between iron indices and lead exposure markers have been found significant in young children (especially in the group of children aged 1-2 years), weaker in older adolescents, and frequently not significant in adults [5,22]. A study focused on the evaluation of lead and iron markers in work-exposed individuals, concluded that blood Pb level was significantly associated with hemoglobin concentration but not with iron status parameters, such as Fe/TIBC, ferritin, and serum iron [23]. Similarly, another study focused on adult subjects, comprising of pregnant women, proved no significant correlations of PbB and iron level [24]. A research trial carried on rats showed that the administration of Pb^{2+} ions caused a significant decrease of serum iron concentration, whereas Fe/TIBC and transferrin levels were increased [25].

As a half-life of Pb body burden is ca. 40 days from intake, it is postulated that PbB concentration may not be the most relevant parameter to evaluate long-term effects of lead exposure [3]. Since lead is known to inhibit ferrochelatase, it results in the accumulation of delta-aminolevulinic acid (ALA) and zinc protoporphyrin (ZPP) in erythrocytes. ZPP reflects the shortage of iron supply in the final stages of hemoglobin synthesis, resulting in Zn insertion into the protoporphyrin molecule [26]. A co-occurrence of Fe depletion and Pb poisoning results in the increase of ZPP level, which consequently leads to inhibition of heme synthesis [5]. The cThe evaluation of mean ZPP levels within the subgroups differing in serum Fe level shown that ZPP was 12.3 % lower ($p = 0.043$) in subjects within the H-Fe subgroup when compared to the L-Fe subgroup. The obtained results were consistent with a former study that found a significant negative association between iron level and ZPP in a cross-sectional study carried on Korean Pb-exposed workers [27]. Elevated levels of erythrocyte ZPP are commonly observed in lead toxicity, together with evidence of increased oxidative stress markers and decreased glutathione peroxidase levels [28].

Due to the fact that iron deficiency and lead poisoning are considered to share common environmental risk factors, it is a standard practice to supplement the diets of Pb-exposed vulnerable populations (especially children) with iron [29,30]. Maintaining the iron level in optimal physiological range is even more important, because both Fe deficiency and increased PbB can contribute to the development of cognitive deficits in children [6,31]. Multiple studies demonstrated positive effects of supplementing iron in the mitigation of lead poisoning in Pb-exposed individuals [29,32–36].

4.3. Correlation of body iron status, lead toxicity and oxidative stress markers

High concentrations of certain metals (physiologically active and/or redundant) are known to be a source of oxidative stress, caused by the generation of reactive oxygen species and inhibition of antioxidant enzymes [37]. Heavy metals, such as lead, covalently interact with sulfhydryl groups of glutathione and enzymes involved in its metabolism (i.a. glutathione peroxidase and glutathione-S-transferase), causing their inactivation [38]. Additionally, the Pb-related inhibition of the activity of other antioxidative enzymes, such as superoxide dismutase (SOD) and catalase (CAT), that are involved in free radicals scavenging, results consequently in increased oxidative stress [7]. Studies on animal models showed the significant effect of lead acetate administration on certain

markers of oxidative stress in the brain tissue [39]. Increased lead concentrations caused a decrease in SOD and GPx activities, as well as the concentration of reduced glutathione (GSH). *In vivo* modeling of dose-dependent effects of lead exposure on serum metabolites was carried in rats [40]. A detailed pathway enrichment analysis revealed the increased abundance of metabolites specifically associated with oxidative stress pathways [40].

The administration of Fe^{2+} (in a form of ferrous sulphate) proved to decrease Pb^{2+} concentrations in the blood and brain tissue and alter the activity of GPx and SOD enzymes and GSH concentration [39]. Some recent studies carried on animal model proved also a causal role of Pb-induced oxidative stress to iron deficiency [8]. Lead exposure caused a significant decrease in the iron absorption level in the midgut of *Drosera melanogaster*. Lead exposure resulted in the increase of reactive oxygen species formation, which thus inhibited Fe^{2+} loading to ferritin by affecting its secretion and trafficking, and consequently impaired iron metabolism [8]. It is postulated that ROS scavenging (i.a. by the administration of antioxidants), can contribute to an increase in the iron uptake (by restoring the expression of iron metabolism genes) and contribute to an alleviation of lead cellular toxicity [8]. The direct supplementation with antioxidants (such as NAC - N-acetyl cysteine) is also recognized as a promising alternative to chelation therapies, even in case of marginal blood lead levels or during constant exposure [41].

Maintaining a proper balance of certain physiologically active metals is known to be an important factor for alleviating Pb-toxicity and mitigating oxidative stress. The correlation of oxidative stress indices of occupationally Pb-exposed subjects has been observed in the case of blood levels of magnesium [42] and calcium [43]. Iron, besides the potential to promote oxidative stress in case of its overload, is an essential element involved in crucial cellular processes, including oxygen transport, transfer of electrons in the respiratory chain and DNA synthesis [44].

Alleviated level of bilirubin can be associated with mitigation of Pb-induced toxicity. A study carried on rats shown an effect of increased bilirubin level on glutathione (GSH) concentration and activation of antioxidative enzymes [45]. A different study that aimed to assess the effects of environmental exposure to Pb toxicity to the general population, exhibited that the increased levels of blood lead and cadmium correlate with increased SOD activity and ferritin concentration [7]. It was also shown that the index of total antioxidant status (TAS) and statutory ferritin level (jointly associated with antioxidative defense) were increased in the population from polluted areas [7]. Another study on occupationally exposed males demonstrated that the increased blood lead levels of subjects was correlated with increased oxidative stress markers (i.e. levels of malondialdehyde and 4-hydroxynonenal) but tested levels of certain antioxidative enzymes, such as superoxide dismutase (SOD) and catalase (CAT), as well as GSH were significantly decreased [46]. A recent study carried on pregnant women with anemia symptoms shown a significant decrease in blood lead levels and a positive effect on antioxidative stress markers after iron supplementation [36]. Measured levels of antioxidant enzymes (CAT and SOD), as well as GSH, were significantly decreased in anemic subjects, while the activity of enzymes recovered after oral admission of iron in form of ferrous sulphate [36].

5. Conclusion

Conducted study on occupationally lead-exposed subjects demonstrated a divergent effect of serum iron level on lead exposure indices. Serum Fe level proved no significant correlation with blood lead levels in the tested population and even slightly higher mean PbB level in the L-Fe group. On the contrary, the H-Fe group was characterized by a lower ZPP level, which is considered a long-term marker related to a hematologic toxic effect of Pb. Thus, low body iron status may enhance an adverse effect of Pb on heme biosynthesis pathways. Interestingly, a carried PCA analysis indicated an existing distinctiveness of examined groups differing in Fe status, proving that individuals classified within the H-Fe group were characterized by a co-occurrence of higher Fe levels, lower ZPP, and higher TAC value. The obtained results may suggest that maintaining the normative status of Fe in the body may play an important role in the prevention of lead poisoning and elimination of the harmful effects of lead on the oxidative balance in humans.

CRediT authorship contribution statement

Michał Słota: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing - original draft. **Marta Wąsik:** Conceptualization, Supervision, Writing - review & editing. **Tomasz Stołtny:** Validation, Writing - review & editing. **Anna Machoń-Grecka:** Conceptualization, Validation, Writing - review & editing. **Aleksandra Kasperczyk:** Data curation, Methodology, Writing - review & editing. **Francesco Bellanti:** Writing - review & editing. **Michał Dobrakowski:** Data curation, Methodology, Writing - review & editing. **Artur Chwalba:** Data curation, Writing - review & editing. **Sławomir Kasperczyk:** Funding acquisition, Supervision, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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