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Impact of Lead (Pb) Exposure on Hematological Parameters, hs-CRP, Ferritin, and Oxidative Stress (MDA) in Battery Factory Workers

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Abstract: Exposure to lead in the workplace is common, with most employers and workers unaware of its adverse health effects. This study examines the relationship between blood lead levels in battery factory workers and health examination results such as Hematology profile (Hemoglobin level, Leukocyte count, Hematocrit Level, Platelet count, Erythrocyte Count, MCV, MCH, and MCHC), malondialdehyde (MDA), ferritin, and hs-CRP. The samples of this study were 25 blood samples of battery factory employees in Jakarta who were older than 40 years and had worked for more than 1 year. Workers' blood samples were collected using vacuum tubes and placed in ice boxes to be sent to PT Petrolab Service Laboratory for blood lead level examination, Citama Hospital Laboratory Unit for blood profile examination, ferritin level, and hs-CRP value, and Prodia Jakarta Clinical Laboratory for MDA level examination. Bivariate analysis examined the relationship between lead levels, blood profile, ferritin levels, MDA levels, and hs-CRP values. Using the entered formula, multivariate analysis was used to test the relationship between lead levels and blood profile, ferritin levels, MDA levels, and hs-CRP values. The significance level used to test the significance of the relationship was <0.05. The bivariate test results showed there was a correlation between blood lead levels and hs-CRP levels (P=0.000), MDA levels (P=0.000), ferritin levels (P=0.000), Hb levels (P=0.000), hematocrit levels (P=0.006), MCV value (P=0.000), and MCH value (P=0.004). In contrast, Multivariate analysis showed lead levels significantly correlated with MDA level (P=0.014), ferritin level (P=0.005), and MCV value (P=0.013). Blood lead levels should be controlled to reduce the risk of oxidative stress and its impact on health, and it is hoped that workers in contact with lead will place more emphasis on occupational safety and health.
Keywords: Blood lead level; ferritin; hematology profile; hs-CRP; workers, malondialdehyde (MDA).

INTRODUCTION

Lead (Pb) contamination of air, soil, and water sources has been attributed to natural processes, such as geochemical weathering, seawater spray emissions, volcanic activity, and remobilization of sediment, soil, and water from mining areas (Cabral Pinto et al., 2020; Cabral Pinto & Ferreira da Silva, 2018; Cabral-Pinto et al., 2020). Anthropogenic products and processes (such as industry, oil processing activities, agrochemicals, paints, smelting, mining, refining, unofficial lead recycling, cosmetics, chipped window and door frames, jewelry, toys, ceramics, pottery, plumbing materials and alloys, water from old pipes, vinyl-containing curtains, stained glass, lead-coated dishes, firearms with lead bullets, batteries, car and truck radiators, and some types of ink) are considered the primary sources of Pb contamination in the environment (Gupta et al., 2019; Hindarwati et al., 2018; A. Kumar et al., 2019; A.

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Kumar & Prasad, 2019; S. Kumar et al., 2019; Kumar Yadav et al., 2018; Lee et al., 2019; Zulfiqar et al., 2019). Pb exerts harmful effects when it enters the human body through ingestion, inhalation, or skin contact (Collin et al., 2022a), is toxic to human health, and is linked to metabolic syndrome (MetS) (Luthviatin et al., 2024).

Lead poisoning is a serious threat to human health, especially for workers who are exposed to lead in their daily activities. It is one of the causes of occupational diseases where workers experience severe complications in several body organs such as kidneys, brain, reproductive organs, and liver (Burnase et al., 2022; Chatha & Naz, 2023). In this cross-sectional study of 6404 people with kidney failure, a low household water lead contamination level was linked to a higher erythropoiesis-stimulating agent dose. Two thousand six hundred forty-eight dari, these patients with advanced chronic kidney disease had lower hemoglobin concentrations (Danziger et al., 2024).

According to the DSM-IV, mixed hyperactive-inattentive ADHD symptoms are associated with blood lead levels below 5 µg/dL. Children with lead levels below 5 µg/dL are more than twice as likely to be diagnosed with ADHD as children whose lead levels are undetectable (Singh et al., 2024). Male employees in the battery industry have been found to suffer hypothermia, testicular atrophy, and low testosterone levels (Queiroz & Waissmann, 2006). The primary cause of male infertility is testicular oxidative stress (Neto et al., 2016). During spermiogenesis, lead binds to human protamines, changing the stability of sperm chromatin and perhaps influencing proper chromatin condensation (Neto et al., 2016; Quintanilla-Vega et al., 2000). By upsetting the balance between antioxidants and reactive oxygen species (ROS), toxicant-induced oxidative stress seriously impairs sperm quality, leading to anomalies in spermatogenesis and male infertility (La Maestra et al., 2015).

Those with aberrant AST, GGT, and total bilirubin had higher blood lead concentrations than the normal population, whereas AST did not significantly differ from the usual population. Grouping the population based on median blood lead levels revealed that the high-concentration group had a considerably larger percentage of those with abnormal AST, GGT, and TBIL than the low-concentration group. The same findings were seen for ALT (Yang et al., 2022). 70% of the world's lead production comes from battery manufacturing and recycling (Dey et al., 2023). Occupational lead exposure is common in developing countries where most employers and workers are unaware of its adverse health effects (Olufemi et al., 2022).

Lead in the body has a half-life of about 30 days in the blood, after which it diffuses into soft tissues such as the kidneys, brain, and liver, then is distributed to bones, teeth, and hair as lead phosphate (Azeh Engwa et al., 2019), with a diffusion process of only 10 g/dL in adults and 1.4 g/dL in children (Okereafor et al., 2020). Lead acts as a cofactor or inhibitor in enzymatic pathways. It is estimated that about half of all enzymes require metal cofactors to be active and functional (Zhang & Zheng, 2020). ROS (reactive oxygen species), such as hydroperoxides, hydrogen peroxide, and singlet oxygen, are produced due to lead poisoning. Pb generates free radicals that cause oxidative stress and can lead to lipid peroxidation. ROS-mediated lipid peroxidation forms various toxic lipid hydroperoxides and lipid aldehydes that act as secondary signal intermediaries in propagating oxidative stress signals that contribute to the pathophysiology of human health and disease (Ramana et al., 2019).

Lead inhibits the activity of 5-aminolevulinic acid dehydratase, resulting in hemoglobin oxidation and lipid peroxidation, which can lead to the hemolysis of red blood cells (Collin et al., 2022). The body experiences oxidative stress when ROS and antioxidant defenses are imbalanced. Oxidative stress leads to cell and tissue damage, which increases the likelihood of adverse health effects such as

cardiovascular disease and cancer (Adeyemi WJ et al., 2020). Polyunsaturated fatty acids are one of the most important molecules directly affected by ROS. Recent studies have also shown that lead exposure-induced ROS formation can negatively impact iron status by interfering with Fe loading onto ferritin (Liu et al., 2020). Several studies have indicated an association between elevated levels of high-sensitivity C-reactive protein (hs-CRP) and exposure to heavy metals such as Cd and Pb (Ravibabu et al., 2019). , but in previous studies, there has been no research linking blood lead levels with hs-CRP, ferritin, and malondialdehyde (MDA) examination parameters at battery manufacturing plants.

Based on the above description, researchers are interested in examining the impact of lead exposure on oxidative stress as seen from health examination parameters. This study also examines the relationship between blood lead levels in battery factory workers and health examination results such as blood profile, malondialdehyde (MDA), ferritin, and hs-CRP. Evaluation of the results of this study is expected to provide a more comprehensive picture of the impact of lead exposure on workers in industrial areas with high levels of lead pollution from the production process.

MATERIALS AND METHODS

This research was approved by the Health Research Ethics Committee of the Muhammadiyah University of Purwokerto No. KEPK/UMP/13/I/2024. A purposive sampling technique was used. Purposive sampling is a technique for determining samples based on specific criteria. The sample criterion was employees aged less than 40 years with a working period of more than 1 year. The samples in this study were whole blood heparin and serum taken from 25 battery factory employees who met the age criteria of 40 years or under and had worked more than 1 year. Sampling was carried out at the Battery Factory in the city of North Jakarta. An explanation of the research was given to respondents before agreeing to take part in the research. The research was carried out using venous blood samples. Workers' blood samples were collected using vacuum tubes and placed in ice boxes to be sent to PT Petrolab Service Laboratory for blood lead level examination, Citama Hospital Laboratory Unit for blood profile examination, ferritin level, and hs-CRP value, and Prodia Jakarta Clinical Laboratory for MDA level examination.

Lead Level Test

Examine blood lead levels using an Agilent 7700 X chemical analyzer with the Inductively Coupled Plasma - Mass Spectrometer (ICP-MS) method, expressing the results in µg/dL units. Reagents for this examination include Argon gas, Helium gas, ICP Multielement Standard Solution VI Certipure, Tuning Solution for ICP-MS 7500 cs, Indium Standard Solution, Mercury Standard Solution, Ammonia Solution 25%, Triton X-100, 1-Butanol, Nitric Acid 65%, Polyethylene, Aqua Ultrapure, HNO3 2%, HCl 0.5%. Before the examination, Whole blood Na Heparin was extracted in an alkaline environment by placing 75µL of Whole blood Heparin in a polypropylene tube, then adding 1425 µL of alkaline solution, and centrifuging at 3000 rpm for approximately 10 minutes, then the supernatant was separated. Argon gas pressure is checked in the range of 500 – 700 kPa, Helium gas pressure is checked in the range of 90 – 130 kPa, and Hydrogen gas pressure is checked in the range of 20 – 60 kPa. Drain lines, cooling water, peristaltic pump tubes, and drying and cleaning tanks are checked. After all preparations have been made, the lead-level test can be started by turning on the Agilent 7700 Series ICP-MS MassHunter software. Then, heating will begin, and a blank solution will be taken for a minimum of 15 minutes. The next stage

after tuning is making a batch by inserting standard, control, and patient samples into the sample tray. After carrying out the test, the analysis results are saved (Susiani & Lestari, 2022).

hs-CRP Level Test

The hs-CRP level test was carried out at the Citama Hospital Laboratory Unit using icroma™ hsCRP all-in-one with the fluorescence immunoassay method, where the results were expressed in mg/dL units. The reagents for hs-CRP examination are Cartridge and Detector Buffer Tube (Catalogue No. CFPC-6, Boditech Med Inc., South Korea). Before the examination, the blood was made into serum by centrifuging for 10 minutes at 2500-3000 rpm after the blood had clotted. 10 µl of serum was taken with the available sample pipette, then placed in a buffer tube, homogenized 10 times by turning it upside down, discarded the first two drops, dripped two drops into the cartridge, and waited 3 minutes. The test cartridge was inserted into the device, and then 'Start' was selected until the test results appeared on the device's monitor screen to be read.

Hematology profile

Hematology profile examination using Mindray BC 5380 Hematology Analyzer to obtain hemoglobin level, leucocyte count, hematocrit level, platelet count, erythrocyte value, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC).

Ferritin Level test

Analysis of ferritin levels using plasma samples from blood specimens centrifuged at 3000 rpm for 15-30 minutes. The instrument is the Ichroma™ II device. Pipette 30 µL of sample to the detector tube. Alternate the tubes 10 times. Pipette 75 µL sample to the test cartridge. Wait 10 minutes, insert the test cartridge into the device, select 'Start,' and read.

Malondialdehyde (MDA) Level test

The examination of malondialdehyde (MDA) levels using plasma samples was then analyzed using the Agilent 1260 tool with the HPLC (High-Performance Liquid Chromatography) method. 100µl of the plasma sample was added to 25µl of 3 M NaOH and incubated in a water bath for 30 minutes at 600C. 1 ml of 0.05 M sulfuric acid and 0.5 ml of TCA 20% b/v were added. The tube was vortexed and centrifuged for 10 minutes at 3000 rpm, then 1 ml of the supernatant was added, 0.5 ml of TBA solution 0.355% b/v, then incubated in a water bath for 40 minutes at 900C, Measurement of MDA through sample preparation with derivatization reagents that convert MDA into fluorescent products, then PH will be optimized by adding 20 µl of supernatant injected into the HPLC system. Separation from HPLC by isocratic method at 30° using reversed-phase column for 4 minutes. Chromatograms were recorded using fluorescence. Quantification was performed with a delivered plasma calibrator, and peak heights were calculated for concentrations using the external standard method.

Data Analysis

The data analysis method uses statistical tests such as univariate bivariate and is carried out with the help of SPSS 25 IBM®SPSS®Statistics software (IBM Corp., Armonk, NY, USA). The data collected were subjected to univariate tests aimed at describing the characteristics of respondents such as age, gender, length of service, compliance with the use of Personal Protection Equipment (PPE), hand washing habits, smoking habits, medical history, and information on the last week of consuming seafood. Data were presented as frequencies and percentages. Bivariate analysis was used to examine the relationship between lead levels and Hemoglobin level,

Leukocyte count, Hematocrit Level, Platelet count, Erythrocyte Count, MCV, MCH, MCHC, ferritin levels, MDA levels, and hsCRP values in Table 2. Using the entered formula, multivariate analysis was used to test the relationship between lead levels and blood profile, ferritin levels, MDA levels, and hsCRP values. The significance level to test the Regression coefficient's significance was <0.05 in Table 3.

RESULTS AND DISCUSSION

The respondents are production workers, ages 22-40, who have worked in production for more than 1 year. Complete respondent characteristics are shown in Table 1.

Table 1. Characteristics of Respondents

Characteristics	N	%
Gender		
Female	4	16
Male	21	84
Blood lead levels (ACGIH, 2023)		
Meets standard (< 20 µg/dL)	12	48
More than standard (≥ 20 µg/dL)	13	52
hs-CRP levels		
Abnormal and low risk (< 1.0 mg/L)	2	8
Abnormal and average risk (1.0 - 3.0 mg/L)	15	60
Abnormal and high risk (> 3.0 mg/L)	7	28
Abnormal and risk of inflammation (10.0 mg/L)	1	4
MDA levels		
Less than Normal (<0.55 mol/L)	0	0
Normal (0.55 - 2.27 mol/L)	25	100
More than normal (> 2.27 mol/L)	0	0
Ferritin Level		
Less than normal (<30 ng/mL)	2	8
Normal (30-350 ng/mL)	6	24
More than normal (>350 ng/mL)	17	68
Hemoglobin level		
Low (Female: <12 g/dL; Male: <14 g/dL)	6	24
Normal (Female: 12-16 g/dL; Male: 14-18 g/dL)	15	60
Increased (Female: >16 g/dL; Male: > 18 g/dL)	4	16
Leukocyte count		
Low (Female: <3600/µL; Male: <3800/µL)	1	4
Normal (Female: 3600-11000/µL; Male: 3800-10600/µL)	24	96
Increased (Female: >11000/µL; Male: >10600/µL)	0	0
Hematocrit Level		
Low (Female: <37%; Male: <42%)	5	20
Normal (Female: 37 – 40%; Male: 42 – 54%)	15	60
Increased (Female: >40%; Male: >54%)	5	20
Platelet count		
Low (<140000 /µL)	0	0
Normal (140000-440000 /µL)	25	100
Increased (>440000/µL)	0	0

Erythrocyte Count		
Low (<4,5 x 10^6/µL)	4	16
Normal (4,5 – 6,5 x 10^6/µL)	20	80
Increased (> 6,5 x 10^6/µL)	1	4
MCV		
Low (<80 fL)	3	12
Normal (80-100 fL)	22	88
Increased (>100 fL)	0	0
MCH		
Low (<26 pg)	3	12
Normal (26-34 pg)	22	88
Increased (> 34 pg)	0	0
MCHC		
Low (<32 %)	2	8
Normal (32-36%)	23	92
Increased (>36%)	0	0

Respondents in this study consisted of 4 women and 21 men with the results of lead levels; 48% still met the ACGIH standard of <20 µg/dL, and 52% exceeded the standard. Ferritin levels exceeded normal by 68%, with abnormal hsCRP levels and inflammatory risk by 4%. Relationship between lead levels and hs-CRP levels, MDA levels, ferritin levels, and blood profile (Table 2).

Table 2. Factors associated with Blood lead levels (BLL)

Variable	Correlation Coefficient	Sig
hs-CRP level	0,997	0,000*
MDA levels	0,984	0,000*
Ferritin Level	0,823	0,000*
Hemoglobin level	-0,658	0,000*
Leukocyte count	0,270	0,192
Hematocrit Level	-0,535	0,006*
Platelet count	0,101	0,632
Erythrocyte Count	-0,311	0,130
MCH	-0,559	0,004*
MCV	-0,893	0,000*
MCHC	0,004	0,985

The bivariate test results showed that there was a relationship between blood lead levels and hs-CRP levels, MDA levels, ferritin levels, Hb levels, haematocrit levels, MCV values, and MCH values (sig<0.05). While for leucocyte count, platelet count, erythrocyte count, and MCHC value showed no association (sig>0.05).

Multivariate test to see the regression of the determinants of hs-CPR lead levels, ferritin levels, and MDA levels with ROS activity as a moderator on the impact of blood lead levels, as well as regression of the determinants of hemoglobin, erythrocytes, leucocytes, hematocrit, platelets, MCV, MCH, and MCHC (Table 3).

The Regression coefficient results showed that blood lead levels significantly correlated with MDA levels, ferritin levels, and MCV values at 5% alpha. The regression coefficients showed that a 1 µg/dL increase in blood lead level would

increase MAD by 585.853 mol/L; ferritin by 0.292 ng/mL, and decrease MCV by 34.841 fL.

Table 3. Regression Coefficient of BLL on hs-CRP, MDA, Ferritin, and Hematology Profile

Variable	Coefficients	Sig
Blood lead levels * hs-CRP level	8,780	0,175
Blood lead levels * MDA levels	585,853	0,005*
Blood lead levels * Ferritin Level	0,292	0,014*
Blood lead levels * Hemoglobin level	-9.172	0,673
Blood lead levels * Leukocyte count	-0.016	0,364
Blood lead levels * Hematocrit Level	1.537	0,791
Blood lead levels * Platelet count	0,001	0,477
Blood lead levels * Erythrocyte Count	12.666	0,768
Blood lead levels * MCH	0,937	0,872
Blood lead levels * MCV	-34,841	0,013*
Blood lead levels * MCHC	20,423	0,351

Blood lead levels showed that 48% of respondents were still within the threshold determined by the American Conference of Governmental Industrial Hygienists (ACGIH) in 2023, namely blood lead levels said to be normal in workers in a work environment that uses lead as a production material is < 200µg/dL, while 52% of mine workers' blood lead levels were more than normal. A meta-analysis study conducted by Olana, A. T., Kumie, A., & Abegaz, T. (2022) from 18 studies of research articles showed the average blood lead level of workers was 37.996 µg/dl (95% CI: 30.680-45.312) which is higher than the threshold value set by the United States government industrial hygiene conference (20 µg/dl) (Olana et al., 2022). Similar research was also conducted by Gomes et al. (2023). A study conducted on workers of a car battery manufacturing and recycling plant showed that the average blood lead level was 21 ± 12 µg/dL and marked above the standard threshold according to ACGIH (Gomes et al., 2023).

The rapid shift towards producing and using clean energy to replace fossil fuels has increased the demand for batteries. Lithium-ion and lead (Pb) batteries have a dominant market share among the available batteries. Lead acid battery plants and smelters rely heavily on lead ore as the primary raw material, with a lead content of more than 70% wet [28]. As with other heavy metals, lead is highly toxic and persistent in the environment, so lead ore processing, including battery production, results in high environmental and health risks [29]. Continuous exposure to heavy metal lead in the body will cause it to accumulate in the body and replace division essential metals such as Ca2+, Mg2+, Fe2+ and unitary cations such as Na+ and ultimately disrupt the biological metabolism of cells, one of which is the replacement of calcium ions by Pb2+(Ciosek et al., 2021; Fu & Xi, 2020).

As Pb concentration increases in the body, the balance between reactive oxygen species (ROS) and antioxidant requirements is disturbed. An increase in ROS ($O2^{\cdot}$, H2O2, $NO^{\cdot}$, $ONOO^{\cdot}$, $OH^{\cdot}$) leads to a decrease in antioxidants. Increased reactive oxygen levels of ROS and Ca2+ in the cell, which in turn leads to decreased mitochondrial potential and apoptosis through the release of cytochrome c. Lead also affects the activity of antioxidant enzymes (Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx)) (Fu & Xi, 2020; Słota et al., 2022; VURAL AYDIN, 2024).

Mitochondria are highly susceptible to oxidative damage due to electrons escaping from the electron transport chain (ETC). The electron transport chain in the inner membrane reacts with oxygen to produce superoxide anion. This anion is unstable and cannot cross the membrane but is quickly converted to hydrogen peroxide, which can penetrate the membrane. It can then undergo the Fenton reaction to generate hydroxyl radicals, which are highly reactive in the mitochondrial matrix. Increased levels of ROS lead to increased mitochondrial DNA (mtDNA) damage, based on which ROS plays an important role in physio- and pathological processes (Juan et al., 2021; Renaudin, 2021; Yan & Zaher, 2019), lipid peroxidation of polyunsaturated fatty acids (such as membrane phospholipids) (Ito et al., 2019); and protein oxidation (Hawkins & Davies, 2019).

Malondialdehyde (MDA) is a marker of oxidative stress; this study showed that blood lead levels correlated with malondialdehyde (MDA). Malik et al. (2020) conducted a study on men exposed in the workplace that showed that increased blood lead levels in the subjects correlated with increased markers of oxidative stress (i.e., malondialdehyde and 4-hydroxynonenal levels). However, levels of certain antioxidant enzymes tested, such as superoxide dismutase (SOD) and catalase (CAT), as well as GSH, decreased significantly (Malik et al., 2020).

Blood lead levels correlate with ferritin levels. ROS formation caused by lead exposure affects the increase in ferritin levels. Research conducted by Wieloch et al. (2012), aimed at assessing the impact of environmental exposure on Pb toxicity in the general population, showed that elevated blood levels of lead and cadmium were correlated with increased SOD activity and ferritin concentrations. It was also shown that the total antioxidant status index (TAS) and ferritin levels (which are jointly associated with antioxidant defenses) increased in populations from polluted areas [40]. ROS will attack the protein ferritin, whose role is to bind and store iron ions in a non-toxic form, by inhibiting the loading of Fe2+ onto ferritin by affecting its secretion and transport and consequently interfering with iron metabolism (Liu et al., 2020), this is due to the formation of reactive free radicals that damage organs due to the process of autooxidation (Tejchman et al., 2021; Wieloch et al., 2012), ferritin here can also be considered as a defense mechanism.

Blood lead levels also correlate with MCV values. Research conducted by Chwalba et al. (2018) showed that chronic lead exposure in the work environment at levels <50 µg/dL does not affect the number of red blood cells and hemoglobin levels but can reduce MCV values, although not significantly. The abnormal MCV state occurs due to the effects of lead exposure; the longer a person is exposed to lead-containing toxic substances, the more the substances that enter the body directly precipitate in high concentrations and can be at risk for increasing blood lead levels. Lead inhibits heme biosynthesis-related enzymes, including ferrochelatase (FECH), coproporphyrinogen oxidase (COIX), and δ-aminolevulinic acid dehydratase (ALAD). Additionally, lead shortens the lifespan of red blood cells and can cause anemia and increased reticulocytosis (Chwalba et al., 2018)

There is an association between blood lead levels and high-sensitivity C-reactive protein (hs-CRP) levels, although no significant correlation exists. Research conducted by Ravibabu et al. (2019) has indicated an association between elevated levels of high-sensitivity C-reactive protein (hs-CRP) and exposure to heavy metals such as Cd and Pb (Ravibabu et al., 2019). This is also similar to the study of Sirivarasai et al. (2013), who found an association between blood lead and hs-CRP and lead and increased systolic blood pressure (Sirivarasai et al., 2013). Lead induces inflammatory mediators that cause damage to vascular endothelial cells (Angeli et al.,

2013). Remarkably, hs-CRP is the most validated inflammatory marker and is widely used as a cardiovascular marker (Nguyen et al., 2021). This study has limitations because it was conducted cross-sectional and only described the results when the research was conducted. No observations were made in the study period. Besides, this study is still not a study because it does not consider all variables, such as the respondent's health history and habits. Take into account all variables, such as the respondent's medical history and the respondent's habits.

CONCLUSION

The correlation test results showed a relationship between blood lead levels and hs-CRP levels, MDA levels, ferritin levels, Hb levels, hematocrit levels, MCV values, and MCH values. Lead levels have a significant effect on MDA levels, ferritin levels, and MCH values. Blood lead levels must be controlled to reduce the risk of oxidative stress and health effects.

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CONFLICT OF INTEREST

There is no conflict of interest related to this research and publication.

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