Vol: 8 Issue: 2

EDJ

Effect of Blood Lead Levels on Hepcidin and other Iron Regulatory Markers in Iron Deficient Pregnant Women

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ABSTRACT

Background and Objectives: Increasing prevalence of pregnancy iron deficiency poses significant health risk that links to the exposure to heavy metals like lead (Pb) which may further complicate this condition. To investigate such a relationship, hepcidin alongside other iron regulatory markers, also, oxidative stress marker Malondialdehyde (MDA) was estimated in relation to Pb in iron deficient (ID) pregnant women.

Methods: The study included 84 pregnant women, blood parameters including hematological, iron markers and blood lead level in ID pregnant women compared with normal pregnant women at 2nd and 3rd trimesters. Lead was measured using ICP- technique, ELISA used for estimation of hepcidin and MDA.

Results: The comparison of the main blood parameters regarding hematological and iron regulatory markers showed significant differences between patient and control groups. Also, blood Pb and MDA concentrations were significantly different. As a feature of lead toxicity, most of the studied blood films revealed that red cell morphology did not exhibit uniformity in size and shape (poikilocytes) which showed significant morphological abnormalities. Pearson correlation analysis revealed statistically significant correlations between Pb level and RBC, HCT, hepcidin, MDA. From the ROC curve analysis of Pb level the result indicated that Pb was a risk factor of ID in patient groups.

Conclusion: The findings suggest an association with significant alterations in hematological parameters and iron metabolism. Concerning the statistical analysis of the ROC curve, as evidenced by the significant correlations observed Pb exposure plays risk factor for ID in pregnant women.

Keywords: Iron deficiency, pregnancy, lead, MDA, hepcidin, ferritin

Article Information

Submission Date: 15/6/2025
Revision date: 10/8/2025
Acceptance date: 29/9/2025
Publishing date: Dec 2025

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DOI: doi.org/10.15218/edj.2025.29

INTRODUCTION

Iron deficiency anemia (IDA) is the most commonly occurring type of anemia, marked by reduced number of red blood cells(RBCs) in circulation due to insufficient iron levels in the bloodstream.[1] Iron is an essential mineral involved in erythropoiesis—the production of RBCs. A deficiency in iron may result from inadequate dietary intake, poor absorption, reduced bioavailability, depleted bodily stores, chronic blood loss, or increased physiological demands.[2] During pregnancy, the demand for iron significantly increases to support maternal blood volume expansion, placental growth, and fetal growth. Failure to meet this elevated demand can lead to maternal complications such as fatigue, heightened susceptibility to infections, and preterm labor.[3] Although iron supplementation is widely recommended during pregnancy, ID individuals may still experience disrupted iron regulation, especially when exposed to environmental xenobiotics like toxic heavy metals.[4] Recent studies highlight the potential for heavy metal exposure such as lead to interfere with iron homeostasis in pregnant individuals. Heavy metals can mimic iron and use the same intestinal receptors, increasing their absorption when iron is deficient.[5] However, limited research exists on how these toxic elements impact iron regulatory mechanisms in pregnant women with ID.[6] Heavy metals, particularly Pb, are pervasive environmental pollutants that pose serious health risks, especially during gestation. Sources include industrial emissions, contaminated food and water, occupational hazards, and daily environmental exposure. These metals are known to disrupt iron metabolism, potentially exacerbating anemia.[7] Despite the importance of this issue, our understanding of the precise mechanisms linking heavy metal toxicity to iron dysregulation remains incomplete.[8] Lead impairs hemoglobin synthesis by inhibiting key enzymatic pathways, endangering both maternal and fetal health. The dual burden of ID and heavy metal exposure represents a critical public health concern. Understanding their interaction is vital for developing effective interventions and policies.[9] This study aims to bridge knowledge gaps by examining the physiological mechanisms through which heavy metals impair iron metabolism, their impact on maternal outcomes, and current methods for exposure assessment.[10] Lead also contribute to oxidative stress via multiple pathways, including the direct generation of reactive oxygen species (ROS)such as production free radicals like MDA.[3] Lead exposure, specifically, is associated with abnormal erythrocyte morphologies such as: Microcytes, hypochromic cells: pale RBCs indicative of low hemoglobin content, target cells (codocytes), chinocytes (crenated cells), RBCs with evenly spaced, small projections.[11] Acantheocytes (spur cells), RBCs with irregular, spiky extensions of varying lengths. Spherocytes: Spherical RBCs lacking the central zone of pallor. Stomatocytes (mouth cells): RBCs with a slit-like area of central pallor.[12]

METHODS

Study Design

The study was conducted on 84 pregnant women at 2nd and 3rd trimesters and were classified into the two groups: Group 1 (Control group): This group composed of 42 healthy pregnant women whose mean age range (25.86 ± 4.78years). They were selected based on a history of no arterial hypertension, cardiovascular, diabetes, renal, lung, central nervous or endocrine system disorders. None of these subjects was under any medical treatment. Group 2 (patient group): This group included 42 ID pregnant women with ages range (25.52 ± 4.733 year). The blood samples were analyzed for the main blood parameters including hematological, iron markers and blood lead level in both groups. CBC was measured by coulter counter machine, lead was measured using inductively coupled plasma mass spectrometry (ICP-MS) technique, ELISA technique was used for estimation of hepcidin and Cobas e 411 was used for ferritin and other markers.

Blood Sample Collection

The blood samples were collected in kargosk Gynecology laboratory and Maternity and children's hospital. Five milliliters (ml) of blood drawn from a vein were collected from the female participants using a disposable plastic syringe and transferred into a 5 ml test tube containing gel and clot activator. The blood was then left to clot and subsequently centrifuged at 1120g for 20 minutes.

Complete Blood Counts (CBC) and Examination of Peripheral Blood Smears

EDTA-treated samples were collected for complete blood count analysis using a multiparameter automated hematology analyzer. To identify blood cell morphology, peripheral blood smears were

stained with Giemsa. Samples were examined using a light microscope (Olympus) at 40x magnification and under oil immersion at 100x magnification. Abnormalities in various parameters were assessed based on their respective standard reference values.

Iron Regulatory Markers

Estimation of Hepcidin was determined by Enzyme-linked Immunosorbent Assay

The Principle of Hepcidin

This ELISA kit employed the sandwich-ELISA technique. The included microelisa strip plate was pre-coated with an antibody specifically targeting Hepcidin (Hepc). Standards or samples were added to the assigned wells of the microelisa strip plate, where they bound to the specific antibody. Subsequently, an antibody specific to Hepc and conjugated with horseradish peroxidase (HRP) It was introduced into each well of the microelisa strip plate and left to incubate. Unbound substances were removed by washing, followed by the addition of TMB substrate solution to each well. Wells that had both Hepc and the HRP-linked Hepc antibody exhibited a blue coloration, which turned yellow after the stop solution was added. The optical density (OD) was subsequently recorded with a spectrophotometer at a 450 nm wavelength. The OD value correlates directly with the concentration of Hepcidin. The Hepcidin levels in the samples were determined by comparing their OD readings to a standard calibration curve (SUNLONG BIOTECH CO.LTD).

Determination of Serum Ferritin Level

The quantification of serum ferritin concentration was conducted utilizing a fully automated immunoassay analyzer (Cobas e 411, Roche Diagnostics, HITACHI, Japan), which operates based on the principles of electrochemiluminescence.[13]

Principle of Electrochemiluminescent

For antibody/antigen-coated micro beads assays were used. Antigen/antibody from the sample combines to the microbead. A microbead with Ab -Ag complex connects the electrode. High voltage current was applied that created an electric field and led to all material in the electrical area to make a reaction. All non-reactive stable precursors produce highly reactive species. Electrochemiluminescent generally used ruthenium complexes (which release a photon at 620nm) regeneration using tripropylamine in either a liquid or liquid-solid phase. It may be used as a monolayer immobilized on an electrode surface. Electrons are excited to higher levels and when they attain stability emission of photons takes place at about 620nm. Photon detection was carried out using photomultiplier tubes, silicon photodiodes, or fiber optic sensors coated with gold.[14]

Determination of Serum Iron and TIBC

Serum iron and TIBC were determined by using the biochemical KENZA analyzer diagnostic kit (4 KENZA 240TX /French).

Estimation of Transferrin Saturation (TS %)

Transferrin saturation was calculated according to Brittenham et al., (2014) as follow:

TS% = (serum iron ÷TBIC) ×100.

Determination of Blood Level of Lead

First the blood sample must be digested before using the ICP method. Blood samples were digested by using microwave methods: Exactly 0.5 ml of whole blood was separately placed into a Pyrex flask. Afterward, 8 ml of a newly prepared blend of concentrated nitric acid and hydrogen peroxide was added (65%–68%) [HNO_3-H_2O_2] was added, and the mixture was left to stand for 10 minutes. The flasks were sealed with watch glasses and subjected to digestion at 150 °C for 10 minutes. The digested specimens were subsequently mixed with 2 ml of nitric acid and several drops of hydrogen peroxide, while heating was continued on a hot plate at approximately 190 °C until a clear solution was formed. The remaining acid mixture was evaporated to a semi-dry residue, allowed to cool, and then diluted with 0.1 ml of nitric acid. The solution was poured into a 100 ml volumetric flask and topped up to the calibration line using distilled water. A blank extraction, using distilled water instead of the sample, was processed through the entire procedure for comparison.[15]

Inductively Coupled Plasma (ICP)

Inductively coupled plasma, which is a sophisticated instrument, was used in determination of Pb concentrations in blood samples based on atomic spectrometry, after due pretreatment. The high-temperature discharge in ICP is produced by passing an electrically conductive gas passing through a magnetic field generated by a load coil encircling the gas-carrying tubes. This elemental analysis method relied on the emission of electromagnetic radiation to identify the elements of interest.

Principle of ICP Analysis Technique

An aerosol of the sample was formed using a suitable nebulizer and spray chamber, then transported into the plasma. In the high-temperature zone

DOI: doi.org/10.15218/edj.2025.29

of the plasma, the sample experienced processes of desolvation, vaporization, atomization, excitation, and ionization. The standard analytical zone refers to the area within the plasma where the emission from the analyte was measured. The element was identified based on its emission wavelength, and its concentration was determined from the intensity of the emission signal.

Determination of MDA was determined by enzyme-linked Immunosorbent Assay

The Principle of MDA

The ELISA kit used in the present was the Sandwish-ELISA method. The Microelisa strip plate provided with this kit was already coated with an antibody that specifically targets MDA. Standards or samples were added to the designated wells, where they bound to the corresponding antibody. Next, an antibody specific to MDA and conjugated with Horseradish Peroxidase (HRP) was added to each well of the Microelisa strip plate and incubated. Unbound substances were then removed by washing. Each well received an addition of the TMB substrate solution. Wells containing both MDA and the HRP-conjugated MDA antibody developed a blue color, which changed to yellow upon the addition of the stop solution. The optical density (OD) was determined using a spectrophotometer at a wavelength of 450 nm. The MDA concentration in the samples was calculated by comparing their OD values to a standard calibration curve (SUNLONG BIOTECH CO.LTD).

Statistical Analysis

Data analysis was conducted using GraphPad Prism 10 and MS Excel 2025, employing descriptive statistical techniques. Continuous data were presented as mean ± standard deviation (SD), Categorical variables were expressed as percentages, and differences between groups were evaluated using the t-test. Normality and lognormality tests to know Parametric or nonparametric and were analyzed by t-test in nonparametric used mann whitney test. Pearson's correlation coefficient (r) was also employed to assess the relationship between the measured parameters and lead (Pb) levels. The Receiver Operating Characteristic (ROC) curve is a graphical representation that plots sensitivity on the y-axis against (100 – specificity) on the x-axis, used to evaluate Pb as a potential risk factor in the ID patient group.

RESULTS

Characteristics of the study participants

Blood parameters between patient group and control groups

The comparison of the main blood parameters between patient and control groups. Levels of hemoglobin HGB(g/dl), hematocrit HCT(%), Average corpuscular hemoglobin MCH(Pg) and average corpuscular hemoglobin concentration MCHC(g/L) were notably reduced in the patient group (10.276±0.970), (33.217 ± 2.983), (23.781 ± 1.768), (58.390 ± 7.420) respectively, when compared to the control group (11.826±0.835), (36.819 ± 2.770), (27.638 ± 2.259), (73.280 ± 11.350) at ($p<0.001$), respectively. Whereas, RBC non significantly decreased in the patient group (4.325±0.410) when compared with control group (4.334±0.452). Furthermore, the patient group had showed a significantly higher mean corpuscular volume (MCV) and red cell distribution width (RDW) levels (84.950 ± 5.593), (73.281 ± 11.352) when compared to control group (76.890 ± 4.183), (58.391 ± 7.427) at ($p<0.001$) as shown in table 1.

Table 1. Comparison of blood parameters between patient and control groups (mean±SD)

Blood parameters	Patient 42	Control 42	P value
RBC 10^6/Ul	4.325 ± 0.410	4.334 ± 0.451	NS
HGB g/dl	10.276 ± 0.970	11.826 ± 0.835	0.001
HCT %	33.217 ± 2.983	36.819 ± 2.770	0.001
MCV fl	84.950 ± 5.593	76.890 ± 4.183	0.001
MCH Pg	23.781 ± 1.768	27.638 ± 2.259	0.001
MCHC g/L	58.390 ± 7.420	73.280 ± 11.350	0.001
RDW-SD fl	73.281 ± 11.352	58.391 ± 7.427	0.001

*RBC: Red blood cell count, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW-SD: Red cell distribution width-standard deviation.

- Data are presented as mean ± standard deviation.
- NS = not significant

Blood level of Pb and iron regulatory markers In this study the distribution of Pb concentrations was significantly different between the control (0.229 ± 0.287) and the patient groups (0.625 ± 0.449) at ($p < 0.001$). Regarding iron markers for both groups, patient group showed significantly higher hepcidin (ng/mL) (54.530 ± 19.007) and ferritin (ng/mL) (56.890± 33.510) compared to the hepcidin: (40.869 ± 11.981) and ferritin (23.500 ± 8.585) for the control group at ($p < 0.001$). Also, Serum iron(µ/dL) levels were significantly higher in the patient group (61.198 ± 20.396) compared to the control group (41.606 ± 13.703) at ($p < 0.001$). Conversely, TIBC(µ/dL) was significantly lower in the patient group (357.394±125.048) compared to the control group (432.502 ± 103.935) at ($p < 0.001$). Transferrin saturation(%) was also significantly lower in-patient group (12.580 ± 5.014) when compared to the control group (15.240 ± 7.267) at ($p < 0.05$) as shown in table 2.

Table 2. Comparison of blood level of Pb and iron regulatory markers between patient and control groups (mean±SD)

Iron markers	Patient 42	Control 42	P value
Pb mg/L	0.625 ± 0.449	0.229 ± 0.287	0.001
Hepcidin ng/mL	54.530 ±9.007	40.869 ± 11.981	0.001
Ferritin ng/mL	56.890± 33.510	23.500± 8.585	0.001
TS %	12.580 ± 5.014	15.240 ± 7.267	0.05
serum iron µ/dL	61.198 ± 20.396	41.606 ± 13.703	0.001
TIBCµ/dL	357.394±125.048	432.502 ± 103.935	0.001

* TS: Transferrin saturation, TIBC: Total iron binding capacity.

Receiver operating characteristics curve (ROC study) for Pb level in patient and control group

ROC curve for Pb level analysis indicated that Pb level was significant at ($P<0.001$) and area under the curve were (AUC= 0.784). The current result indicates that Pb was a risk factor of iron deficiency in patient groups as shown in figure 1.

Correlation coefficient (r) between data

correlation between Pb and blood parameters

Lead was related significantly with blood parameters Correlation analysis revealed statistically significant correlations between Pb and (RBC; r= 0.357, p=0.020), (HCT; r=-0.309, p=0.046). while there was no significant correlation between Pb and (HGB; r=−0.044, p=0.779), (MCV; r=0.002, p=0.989), (MCH; r=0.058, p=0.714), (MCHC; r=0.063, p=0.691), or (RDW; r=0.021, p=0.894) as shown in table 3.

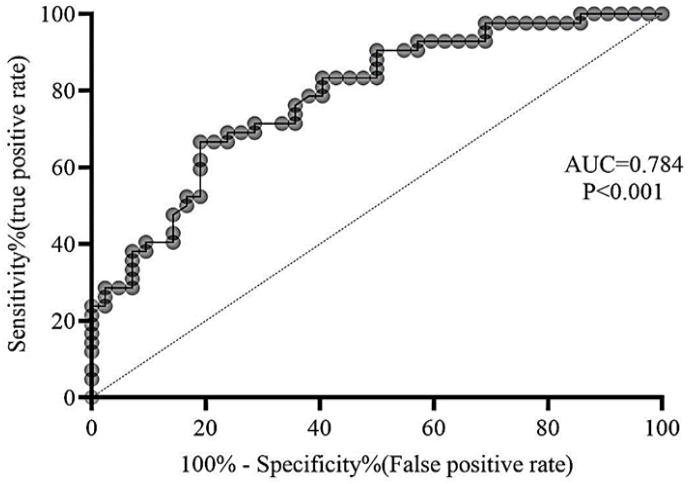


Figure 1 The ROC curve shows the sensitivity and specificity of Pb level in patient and control groups. AUC: Area under the curve

DOI: doi.org/10.15218/edj.2025.29

Table 3. Pearson's correlation between Pb level and blood parameters

		RBC10^6/ uL	HGB g/ dl	HCT %	MCV fl	MCH Pg	MCHC g/ L	RDW fl
Pb mg/L	r	0.357	-0.044	-0.309	0.002	0.058	0.063	0.021
	p	0.02	0.779	0.046	0.989	0.714	0.691	0.894

Association between Pb level and iron regulatory markers, oxidative stress MDA

Pearson's Correlation analysis revealed a statistically significant positive correlation between Pb and hepcidin (r=0.317, p=0.05), as well as a significant positive correlation between Pb and MDA (r=0.360, p=0.018). No other statistically significant correlations were observed between lead and ferritin (r=0.252,p=0.107), serum iron (r=0.115,p=0.466), TS (r=0.124,p=0.432), or TIBC (r=−0.010, p=0.949) as shown in table 4.

Table 4. Pearson's correlation between Pb level with iron regulatory markers and oxidative stress

Parameters		Hepcidin ng/mL	Ferritin ng/mL	Serum iron ng/mL	TS %	TIBC µ/dL	MDAµ/dL
Pbmg/L	r	0.317	0.252	0.115	0.124	-0.010	0.360
	p	0.05	0.107	0.466	0.432	0.949	0.018

Effect of Pb on RBC morphology

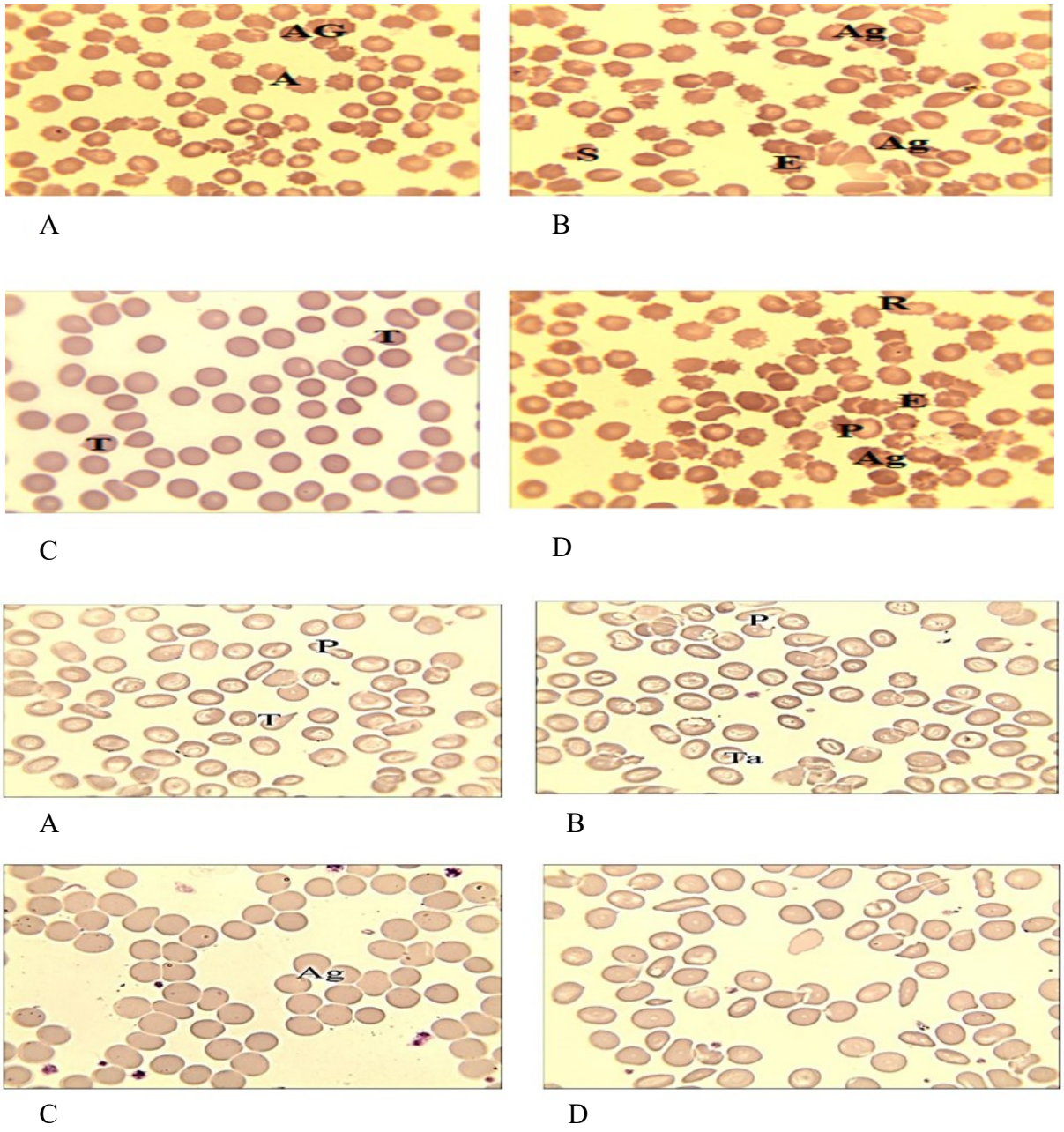
As shown in figure 2 (A, B, C, and D) illustrating the morphology of RBCs, potentially influenced by Pb exposure.

figure A. shows a field of abnormal-appearing red blood cells. The cells are predominantly biconcave discs, but they appear round with a characteristic central pallor, consistent with healthy erythrocytes. They did not exhibit uniformity in size and shape.

figure B. depicts red blood cells exhibiting significant morphological abnormalities. A substantial proportion of the cells are spiculated, displaying irregular, thorny projections on their surface. These cells are consistent with acanthocytes (also known as spur cells) or possibly severe echinocytes, which can indicate altered membrane integrity. The presence of such poikilocytes suggests a significant disruption to normal erythrocyte physiology, which can be a feature of lead toxicity.

figure C. presents red blood cells that appear largely normal, similar to those in Panel A. The cells maintain their typical biconcave disc shape and exhibit appropriate central pallor,

figure D. further illustrates red blood cells with prominent spiculation, similar to those observed in Panel B. A considerable number of cells show an irregular, thorny, or crenated appearance, strongly indicative of acanthocytic or echinocytic transformation. If these samples are from lead -exposed subjects, these morphological changes suggest the detrimental effect of lead on red blood cell membrane structure and function.



*T: Tear cell, A: Acanthocyte, E: Echinocyte, Ag: Aggregation of RBCs, P: Pencil shape, R: rod shape, S: schistocyte, Ta: Target cell.

Figure 2. Peripheral blood smear showing microcytic, hypochromic red blood cells in ID patients, (100X, magnification)

DISCUSSION

The study reveals significant differences in hematological parameters and iron regulatory markers between the patient and control groups, alongside elevated blood Pb levels in the patient group. Specifically, patients exhibited lower levels of HGB, HCT, MCH, and MCHC, suggestive of a potential iron-deficient state. Conversely, MCV and RDW levels were elevated in the patient group. However, there was no significant difference in RBC counts between the two groups, these findings collectively point to alterations in red blood cell production and morphology in the patient group.[16] Furthermore, patients

DOI: doi.org/10.15218/edj.2025.29

showed significantly increased levels of hepcidin and ferritin, along with decreased TS, TIBC all consistent with ID. Paradoxically, serum iron levels were significantly elevated in the patient group. This apparent contradiction of elevated serum iron in the presence of other iron deficiency markers—may be attributable to the disruptive effects of lead exposure on iron metabolism.[9] The markedly higher lead concentrations observed in the patient group, corroborated by ROC curve analysis identifying lead as a potential risk factor for iron deficiency, underscore the possible role of lead in impairing iron homeostasis. Lead and iron share common gastrointestinal absorption pathways, primarily via the Divalent Metal Transporter1(DMT1), a non-specific metal transporter. Under iron-deficient conditions, DMT1 expression is upregulated in the duodenum to enhance iron uptake. However, this compensatory mechanism also facilitates increased absorption of other divalent metals, such as lead, when present in the environment or diet.[17] Although correlation analysis did not reveal statistically significant associations between lead levels and most individual hematological parameters, a significant positive correlation was found between lead and both hepcidin and ferritin, as well as between Pb and MDA, an oxidative stress marker. These findings suggest that lead exposure may influence iron regulation through effects on hepcidin expression and by inducing oxidative stress, although the exact mechanisms remain to be elucidated.[18]

Lead disrupts iron homeostasis through multiple mechanisms, including competitive inhibition of iron absorption and interference with heme biosynthesis. These disruptions can result in functional iron deficiency or exacerbate pre-existing IDA. In response to ID, the liver reduces hepcidin production to enhance iron absorption and mobilization from stores.[19] Because lead impairs iron utilization for hemoglobin synthesis, it mimics or induces a functional ID, thereby indirectly contributing to the physiological cues that suppress hepcidin production. Consequently, lead may aggravate the very deficiency that the body attempts to correct via reduced hepcidin expression.[20]

Elevated MDA levels reflect increased lipid peroxidation, the oxidative degradation of lipids, particularly in cell membranes, resulting in cellular damage. Lead, which has no physiological role in humans, acts as a potent pro-oxidant.[21] It can directly ROS such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide. These ROS attack cellular structures, including membrane lipids. In pregnant women with IDA, concurrent lead exposure may amplify oxidative stress through a synergistic mechanism: lead directly promotes ROS generation and undermines antioxidant defenses, while IDA increases vulnerability to oxidative damage due to impaired red cell integrity and altered iron handling. This combined burden likely contributes to the pronounced elevation in MDA levels observed, signifying increased oxidative damage.[22] The absence of significant correlations between lead and certain iron markers (serum iron, TS, TIBC) highlights the complexity of lead's interaction with iron metabolism and warrants further investigation. Overall, the findings suggest that elevated lead levels are associated with altered hematological profiles and dysregulated iron homeostasis, potentially contributing to iron deficiency in the affected patient group.[23]

CONCLUSIONS

The findings of this study highlight a significant association between elevated blood Pb levels and altered hematological and iron regulatory profiles suggesting a contributory role of lead exposure in the pathogenesis of IDA. Potentially mediated by lead toxicity. Also, the significant positive correlations between Pb with hepcidin and MDA, suggest that lead may exert its effects not only by interfering with iron metabolism but also by enhancing oxidative damage.

Moreover, the ROC analysis confirmed Pb as a potential risk factor for IDA, reinforcing the need to consider environmental toxicants in the evaluation and the complex interplay between heavy metal exposure and iron metabolism, highlighting the importance of screening for environmental toxins in ID individuals, particularly in high-risk groups.

ACKNOWLEDGMENTS

The author would like to thank the private Hemn laboratory Mr. Hemn Salh Kareem and to Dr. Ahamd Hassan for his continuous advises and assistances.

CONFLICT OF INTEREST

There no conflict of interest in this work. This study has not been supported by any individual and /or organizations

FUNDING

No funding.

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DOI: doi.org/10.15218/edj.2025.29