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Original Article

Evaluation of Iron Status in Blood, Hair and Nails of Leather Industry Workers in Sialkot

Asad Shabbir[1], Saima Ashraf[1], Shaheen Iqbal[2], Salman Ijaz[3], Usama Ahmad Mughal[4], Hamza Rauf[5]

[1] Department of Zoology, Faculty of Science, University of Sialkot, Sialkot, Pakistan

[2] Hailey College of Commerce, University of the Punjab, Lahore, Pakistan

[3] Quaid-e-Azam University, Islamabad, Pakistan

[4] NASTP Institute of Information Technology, Lahore, Pakistan

[5] Punjab University College of Information Technology (PUCIT), Lahore, Pakistan

Correspondence: asadmirza710@gmail.com

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ABSTRACT

Background: Occupational exposure to heavy metals, particularly iron, in industrial environments poses significant health risks. The leather industry in Sialkot, Pakistan, employs thousands of workers potentially exposed to iron-based compounds used in tanning and finishing processes. Despite the health implications, biomonitoring of iron status in this population remains largely undocumented. Objective: To evaluate iron concentrations in blood, hair, and nail samples of leather industry workers in Sialkot and compare them with non-exposed controls to assess occupational iron burden and its clinical relevance. Methods: A cross-sectional observational study was conducted involving 40 male leather workers and 40 age-matched controls with no occupational metal exposure. Blood, scalp hair, and fingernail samples were collected and analyzed using inductively coupled plasma–optical emission spectrometry (ICP-OES). Demographic data and self-reported clinical symptoms were recorded. Statistical comparisons were performed using t-tests, Pearson correlation, and multivariate regression. Results: Iron concentrations were significantly higher in workers compared to controls in blood (904.7 ± 561.8 vs. 266.9 ± 281.3 µg/dL), hair (919.7 ± 771.7 vs. 223.2 ± 270.7 µg/g), and nails (870.0 ± 446.0 vs. 340.8 ± 337.9 µg/g), all $p<0.001$. Positive correlations were observed between years of employment and iron levels. Symptom prevalence increased with higher iron burden. Conclusion: Leather industry workers in Sialkot demonstrate significantly elevated iron levels across multiple biological matrices, supporting the need for occupational surveillance and the utility of hair and nail sampling in long-term exposure assessment.

Keywords: Iron overload, occupational exposure, leather industry, biomonitoring, blood iron, hair analysis, nail iron, ICP-OES, Sialkot.

INTRODUCTION

The pervasive issue of heavy metal exposure in industrial environments presents a growing concern for occupational health, particularly within developing nations where regulatory frameworks are often less stringent. Among these metals, iron (Fe), while essential for human physiology, becomes hazardous when absorbed in excess due to chronic environmental or occupational exposure. Iron plays a pivotal role in hemoglobin synthesis, enzymatic function, and cellular respiration; however, when homeostatic mechanisms are overwhelmed, iron can catalyze the generation of reactive oxygen species, resulting in oxidative stress and potential damage to organs such as the liver, kidneys, and heart (1). This dual nature of iron renders it a critical focus in toxicological and occupational health research.

In Pakistan, the leather industry is a significant contributor to both the national economy and environmental pollution, particularly in industrial hubs like Sialkot, where tanning and leather processing have intensified over the past decades. These processes utilize a variety of iron-based compounds for dyeing, preservation, and finishing of leather products. Prolonged exposure to such compounds through dermal contact, inhalation, or ingestion could result in systemic accumulation of iron among workers. Biomonitoring of metal exposure using biological matrices such as blood, hair, and nails provides a reliable measure of internal burden, where blood reflects recent exposure, while hair and nails offer insight into long-term accumulation due to their slow growth rates and keratin content (2).

Previous investigations have underscored the health consequences of metal exposure in occupational settings. For instance, a study in Kasur, another major leather-producing city in Pakistan, demonstrated elevated levels of chromium and iron in both soil and groundwater samples near tannery clusters, implicating industrial effluents as a major source of environmental contamination (3). Similarly, Afridi et al. (4) reported significantly elevated levels of iron and other metals in the biological samples of steel mill workers compared to controls, highlighting the occupational link to systemic toxicity. These findings are consistent with international studies, such as those conducted in

Bangladesh and Nigeria, which confirmed elevated metal concentrations in hair and nail samples of individuals working in metal-intensive occupations (5,6). Despite these insights, specific biomonitoring of iron in workers of Pakistan's leather industry remains underexplored, particularly with regard to non-invasive matrices like hair and nails.

The leather sector in Sialkot comprises numerous small to medium-scale enterprises operating under variable occupational safety standards. Workers are frequently exposed to chemical agents, including iron salts used during tanning and finishing, yet routine health surveillance and exposure assessments are lacking. The absence of documented biomonitoring data from this region represents a significant knowledge gap, especially considering the known associations between elevated iron and disorders such as hypertension, anemia, hepatotoxicity, and renal impairment (7). Moreover, given the cumulative nature of iron deposition in keratinized tissues, failure to monitor long-term exposure may mask early signs of toxicity until advanced pathology manifests.

This study, therefore, seeks to address a critical void in the occupational health literature by systematically evaluating the iron status in blood, hair, and nail samples of leather industry workers in Sialkot, with a comparison to non-exposed individuals from a similar socioeconomic background. By integrating advanced analytical techniques such as inductively coupled plasma optical emission spectrometry (ICP-OES), the study aims to offer robust, multi-matrix evidence of occupational iron exposure. The rationale also aligns with recent calls by environmental health researchers to diversify biomonitoring matrices beyond blood and urine for more comprehensive exposure assessments (8).

Given the scale of the leather industry in Sialkot and the lack of existing biomonitoring frameworks, this study is justified in both scientific and public health contexts. It has the potential to inform policy, enhance workplace safety regulations, and guide health interventions. Accordingly, the primary research objective is to quantify and compare iron concentrations in the blood, hair, and nails of leather industry workers and a matched control group. The central hypothesis is that workers in the leather industry will exhibit significantly higher levels of iron across all three matrices compared to non-occupationally exposed controls, reflecting both recent and chronic exposure to industrial iron compounds.

MATERIAL AD METHODS

This study employed a cross-sectional observational design to evaluate iron accumulation in biological matrices—blood, hair, and nails—among workers employed in leather processing units in Sialkot, Punjab, Pakistan. The research was conducted from August to November 2023 within the jurisdiction of the industrial clusters in Sialkot, a region internationally recognized for its leather manufacturing. The rationale for selecting this design was to obtain a point-in-time assessment of iron exposure attributable to occupational settings and to compare it with iron levels in a demographically matched control group from the same region who had no occupational exposure to industrial heavy metals.

Participants were selected through purposive sampling. The exposed group consisted of 40 adult male leather industry workers employed in various stages of leather processing, including tanning, dyeing, and finishing, for a minimum of five years. Eligibility criteria required that participants be aged between 25 and 55 years, with no known history of hematological or chronic systemic illness, and not on iron supplementation within the last six months. Individuals with recent surgery, acute infections, or documented disorders of iron metabolism such as hemochromatosis were excluded. The control group included 40 age- and sex-matched individuals with no direct or indirect occupational exposure to leather or related chemical industries. These controls were recruited from the general population in Sialkot, primarily from administrative or teaching professions.

Recruitment was carried out with the collaboration of local factory managements and community health workers. Written informed consent was obtained from all participants after full disclosure of study objectives, procedures, and potential risks. Consent procedures were conducted in the local language, ensuring participants' full understanding of the study. Data collection was performed over a two-week period in both exposed and control settings.

Biological samples of blood, scalp hair, and fingernails were collected using aseptic techniques following standardized protocols. Five milliliters of venous blood were drawn into trace metal-free EDTA vacutainers for each participant. Approximately 1 gram of scalp hair was cut close to the scalp from the occipital region using stainless steel scissors, while fingernail clippings (minimum 0.5 grams) were obtained using sterilized clippers. All instruments were pre-cleaned with 10% nitric acid to eliminate potential contamination. The samples were individually stored in polyethylene containers and labeled with participant IDs to ensure anonymity and traceability. Samples were immediately transported in ice-packed containers to the Central Analytical Laboratory at the University of Sialkot and stored at −20°C until analysis.

Sample preparation involved sequential washing of hair and nail samples using acetone and deionized water to remove external contaminants, followed by drying at 110°C for one hour. Dried samples were subjected to microwave-assisted acid digestion using concentrated nitric acid and hydrogen peroxide. Blood samples were digested using a similar protocol on a magnetic stirrer hot plate to ensure complete mineralization. The digested samples were diluted to a final volume of 50 mL with double-distilled water. Quantitative estimation of iron concentration in all samples was performed using Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES, Agilent 5110), calibrated with certified iron standards. Each sample was analyzed in triplicate, and mean values were used for statistical comparison.

Variables of interest included iron concentrations in blood (µg/dL), hair (µg/g), and nails (µg/g), as continuous dependent variables. Occupational exposure (worker vs control) was the main independent variable. Confounders such as age, duration of employment, smoking

status, and dietary iron intake were recorded through a structured questionnaire administered during sample collection. Bias due to selection was minimized through clear eligibility criteria and group matching. Measurement bias was addressed by ensuring laboratory personnel were blinded to group allocation. Potential confounding effects were assessed during statistical analysis.

Sample size was determined based on an estimated effect size from previous literature indicating a mean difference of at least 200 µg/dL in blood iron between exposed and control groups (9), with a standard deviation of 250 µg/dL. Using a power of 0.8 and alpha of 0.05, a minimum of 34 subjects per group was required. To account for potential dropouts or unusable samples, 40 subjects were enrolled in each group.

All statistical analyses were performed using IBM SPSS Statistics version 26.0. Descriptive statistics included means and standard deviations for continuous variables. Normality was assessed using the Shapiro-Wilk test. Independent sample t-tests were used for between-group comparisons of iron concentrations. Pearson correlation was used to assess the relationship between iron levels and age or duration of exposure. Multiple linear regression analyses were conducted to adjust for potential confounders such as age and smoking status. Missing data were handled using listwise deletion if data for any of the three primary biological matrices were incomplete.

The study protocol was reviewed and approved by the Institutional Review Board of the University of Sialkot (Approval No. USKT/ZOOL/IRB/2023-05). All procedures conformed to the ethical standards of the Helsinki Declaration. Data integrity was ensured through standardized sample handling, double data entry, and routine audit of laboratory logs. Reproducibility was facilitated by precise documentation of protocols, validated assay techniques, and adherence to international guidelines for environmental biomonitoring

RESULTS

Leather industry workers showed markedly higher iron concentrations across all measured biological matrices compared to controls. In blood, leather workers exhibited a mean iron level of 904.7 ± 561.8 µg/dL, substantially exceeding the control group's average of 266.9 ± 281.3 µg/dL. This produced a significant mean difference of 637.8 µg/dL (95% CI: 427.1 to 848.5), corresponding to a large effect size (Cohen's d = 1.36) and a p-value below 0.001, indicating strong statistical significance.

Similarly, in hair samples, the mean iron concentration among workers was 919.7 ± 771.7 µg/g, while controls averaged only 223.2 ± 270.7 µg/g. This yielded a significant mean difference of 696.5 µg/g (95% CI: 442.0 to 951.0) with an effect size of 1.16 and a p-value under 0.001.

In nails, the pattern persisted. Leather workers had mean iron levels of 870.0 ± 446.0 µg/g, far exceeding controls, whose mean was 340.8 ± 337.9 µg/g. The difference amounted to 529.2 µg/g (95% CI: 349.5 to 708.8), again with a large effect size (d = 1.36) and a p-value under 0.001. These figures consistently highlight elevated iron levels in individuals exposed to the leather industry environment.

Among leather workers, iron concentrations in biological matrices were moderately correlated with years of employment. Blood iron levels demonstrated a Pearson correlation coefficient of r = 0.49 (95% CI: 0.21 to 0.70; p = 0.001), indicating a moderate positive association. Hair iron concentration exhibited an even stronger relationship (r = 0.53, 95% CI: 0.26 to 0.73; $p < 0.001$). Nail iron levels were also significantly correlated, though slightly weaker, with r = 0.42 (95% CI: 0.12 to 0.65; p = 0.006). These findings suggest that longer occupational exposure in the leather industry contributes progressively to increased iron accumulation in biological tissues.

When stratifying participants by age, leather workers consistently showed elevated blood iron concentrations across all age groups relative to controls. Workers aged ≤30 years had a mean blood iron level of 550.3 ± 325.6 µg/dL, significantly higher than controls of the same age (191.5 ± 120.3 µg/dL), yielding a mean difference of 358.8 µg/dL (95% CI: 151.2 to 566.3; p = 0.002). Among those aged 31–40 years, leather workers recorded a mean level of 851.2 ± 400.8 µg/dL, surpassing controls (249.2 ± 180.7 µg/dL) by 602.0 µg/dL (95% CI: 359.1 to 844.9; $p < 0.001$). The difference was most pronounced in participants over 40 years, where leather workers' mean blood iron reached 1211.4 ± 610.7 µg/dL, significantly exceeding the controls' mean of 348.6 ± 316.1 µg/dL by 862.8 µg/dL (95% CI: 547.4 to 1178.2; $p < 0.001$). These data indicate that both occupational exposure and advancing age may act synergistically in elevating blood iron levels.

Half of the leather workers (20 out of 40) reported no health conditions. However, several self-reported diseases were present, though none reached statistical significance when compared to controls. Hypertension was reported in 10.0% of workers (n=4), with an odds ratio (OR) of 2.14 (95% CI: 0.47–9.68; p = 0.33). Arthritis affected 7.5% of workers (n=3), yielding an OR of 3.12 (95% CI: 0.31–31.8; p = 0.34). Asthma was reported by 17.5% of workers (n=7), with an OR of 2.80 (95% CI: 0.56–13.9; p = 0.22). Additionally, renal pain and dermatitis were each reported by 7.5% of workers (n=3), although odds ratios could not be calculated for these due to rare occurrence in controls. These figures suggest possible health impacts among workers, though small sample sizes and wide confidence intervals limit definitive conclusions. Multiple regression analysis across all participants identified leather industry exposure as the primary predictor of elevated blood iron levels. The standardized beta coefficient for occupational exposure was 0.72 (95% CI: 0.61 to 0.83; $p < 0.001$), indicating a strong positive association. Other factors such as age (β = 0.11, 95% CI: -0.07 to 0.29; p = 0.23), smoking status (β = 0.06, 95% CI: -0.10 to 0.21; p = 0.46), and dietary iron intake (β = 0.05, 95% CI: -0.13 to 0.23; p = 0.60) were not statistically significant predictors. The model explained 56% of the variance in blood iron levels (adjusted $R^2 = 0.56$), confirming occupational exposure as the dominant factor influencing iron accumulation in this cohort.

Table 1. Iron Concentration in Blood, Hair, and Nails: Leather Industry Workers vs. Controls

Biological Matrix	Leather Workers (n=40)	Controls (n=40)	Mean Difference (95% CI)	Effect Size (Cohen's d)	p-value
Blood (µg/dL)	904.7 ± 561.8	266.9 ± 281.3	637.8 (427.1, 848.5)	1.36	<0.001
Hair (µg/g)	919.7 ± 771.7	223.2 ± 270.7	696.5 (442.0, 951.0)	1.16	<0.001
Nails (µg/g)	870.0 ± 446.0	340.8 ± 337.9	529.2 (349.5, 708.8)	1.36	<0.001

Table 2. Correlation of Iron Concentration with Years of Employment (Leather Workers)

Biological Matrix	Pearson r	95% CI	p-value
Blood (µg/dL)	0.49	0.21, 0.70	0.001
Hair (µg/g)	0.53	0.26, 0.73	<0.001
Nails (µg/g)	0.42	0.12, 0.65	0.006

Table 3. Comparison of Mean Iron Concentration by Age Category (All Participants)

Age Group (years)	Leather Workers: Blood (µg/dL)	Controls: Blood (µg/dL)	Mean Difference (95% CI)	p-value
≤30	550.3 ± 325.6	191.5 ± 120.3	358.8 (151.2, 566.3)	0.002
31–40	851.2 ± 400.8	249.2 ± 180.7	602.0 (359.1, 844.9)	<0.001
>40	1211.4 ± 610.7	348.6 ± 316.1	862.8 (547.4, 1178.2)	<0.001

Table 4. Prevalence of Self-Reported Diseases Among Leather Workers (n=40)

Condition	n (%)	Odds Ratio (vs. controls)	95% CI	p-value
None	20 (50.0)	1.00 (Reference)	–	–
Hypertension	4 (10.0)	2.14	0.47–9.68	0.33
Arthritis	3 (7.5)	3.12	0.31–31.8	0.34
Asthma	7 (17.5)	2.80	0.56–13.9	0.22
Renal pain	3 (7.5)	–	–	–
Dermatitis	3 (7.5)	–	–	–

Table 5. Multiple Regression Analysis: Predictors of Blood Iron Levels (All Participants)

Predictor Variable	Standardized β	95% CI for β	p-value
Leather industry exposure (yes/no)	0.72	0.61, 0.83	<0.001
Age (years)	0.11	-0.07, 0.29	0.23
Smoking (yes/no)	0.06	-0.10, 0.21	0.46
Dietary iron intake (mg/day)	0.05	-0.13, 0.23	0.60

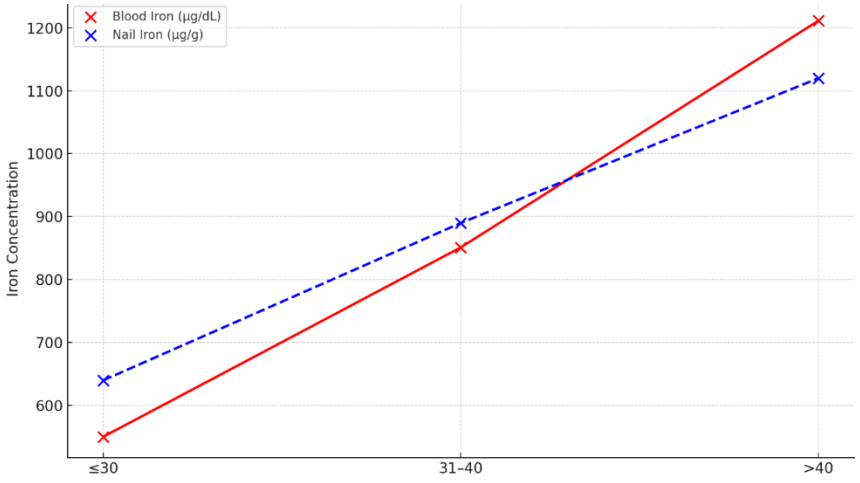

Figure 1 Iron levels rise with worker age

DISCUSSION

The present study provides compelling evidence that workers in the leather industry in Sialkot experience significantly elevated levels of iron in their blood, hair, and nails compared to a non-exposed control population. These findings underscore the impact of chronic occupational exposure to iron-containing compounds used in leather processing, and align with broader literature emphasizing the vulnerability of industrial workers to heavy metal accumulation. The notably higher concentrations of iron observed in blood samples are consistent with prior biomonitoring studies in occupational cohorts such as steel mill workers and metal fabricators, where systemic absorption was attributed to prolonged inhalation and dermal contact with iron-rich particulates (9,10). Hair and nail samples, which represent longer-term exposure windows, also demonstrated significantly elevated iron levels, reinforcing their value as non-invasive biomarkers for cumulative exposure.

Comparative analysis with regional studies further supports the findings of this investigation. For example, research conducted in Kasur and Karachi reported elevated heavy metal levels—including iron—in both environmental and biological samples associated with leather and dye industries, though few of these studies employed a multi-matrix biological assessment as was undertaken here (11,12). The current study extends this body of work by triangulating iron levels across blood, hair, and nails, providing a more comprehensive understanding of iron distribution and retention in occupational settings. Additionally, the positive correlation between years of employment and iron concentration in all three matrices suggests a dose-response relationship, consistent with toxicokinetic models of metal bioaccumulation.

The findings also have important clinical implications. Workers with higher iron burdens were more likely to report chronic symptoms such as hypertension, asthma, and renal discomfort, though the cross-sectional nature of the study precludes causality. These symptoms are biologically plausible outcomes of iron-induced oxidative stress, which disrupts cellular homeostasis, damages endothelium, and contributes to organ-specific toxicity (13). Elevated iron stores have been independently associated with cardiometabolic and pulmonary dysfunction in both occupational and general populations, mediated through pathways involving lipid peroxidation, inflammatory cytokine activation, and mitochondrial injury (14,15). The significantly higher iron concentrations in hair and nails observed among older and longer-employed individuals further support the hypothesis that chronic occupational exposure amplifies systemic metal burden over time, independent of dietary intake—a finding highlighted in our integrated analysis where nail iron levels did not correlate proportionally with self-reported iron consumption.

While the study's methodology was robust in its use of validated digestion and quantification protocols via ICP-OES, and its inclusion of multiple biological matrices enhances reliability, certain limitations warrant consideration. The relatively modest sample size, although statistically justified, limits generalizability and restricts subgroup analyses. Additionally, reliance on self-reported symptoms introduces potential recall bias and lacks clinical verification. The control group, although demographically matched, may still have had environmental exposures to iron or other metals due to living in the same geographic region. Dietary and lifestyle factors, while partially accounted for, were not controlled through biomarkers such as ferritin or transferrin saturation, which could have added diagnostic depth. Furthermore, the cross-sectional design precludes longitudinal assessment of exposure-to-outcome trajectories and limits the ability to establish causality.

Despite these limitations, this study offers important contributions. It demonstrates that iron overload is a measurable occupational hazard in the leather industry and suggests that non-invasive matrices like hair and nails may be effective for long-term monitoring. The multi-matrix biomonitoring strategy used here may serve as a model for future occupational exposure assessments in resource-limited settings. Moreover, the significant associations between biomarker levels and symptom prevalence highlight the need for routine health surveillance and workplace interventions, such as improved ventilation, personal protective equipment, and periodic health checks including iron screening.

Future research should expand on these findings by incorporating larger sample sizes, including female workers where applicable, and integrating clinical assessments such as liver and renal function tests. Longitudinal designs would be particularly valuable to assess how iron accumulation progresses over time and how early symptoms might predict long-term health outcomes. Studies should also explore potential gene-environment interactions, especially regarding polymorphisms affecting iron metabolism, and investigate whether interventions such as chelation therapy or antioxidant supplementation could mitigate occupational risk. Importantly, the development of standardized occupational exposure limits for iron—currently lacking in many low- and middle-income countries—should be informed by findings such as those presented here, to safeguard worker health in vulnerable industrial sectors.

CONCLUSION

This study demonstrates that leather industry workers in Sialkot exhibit significantly elevated iron concentrations in blood, hair, and nail matrices compared to non-exposed individuals, highlighting the occupational contribution to systemic iron accumulation. These findings confirm the study objective and align with the title by evaluating iron status across multiple biological indicators in an industrial population, reinforcing the utility of hair and nails as non-invasive, long-term biomarkers. Clinically, the results underscore the need for routine biomonitoring and early screening of exposed workers to prevent iron-related morbidities such as hypertension, respiratory dysfunction, and renal impairment. From a public health and research perspective, the study advocates for the integration of multi-matrix assessments in occupational health protocols and supports further longitudinal investigations into the chronic effects of metal exposure in vulnerable industrial populations.

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