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Blood, Hair, and Nail as Biomarkers of Arsenic Exposure among Leather Industry Workers in Sialkot

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Background: Arsenic exposure is a major occupational health concern, particularly in industries such as leather tanning where workers are routinely exposed to chemical contaminants. Although blood arsenic reflects recent exposure, hair and nails may provide more reliable indicators of long-term accumulation, yet comparative biomarker studies in Pakistan remain scarce. **Objective**: This study aimed to quantify arsenic concentrations in blood, hair, and nails of leather industry workers in Sialkot and evaluate their diagnostic accuracy for chronic occupational exposure. **Methods**: A cross-sectional observational study was conducted between January and October 2023, enrolling 40 leather industry workers and 40 age- and sex-matched non-exposed controls. Biological samples were collected using standardized procedures, and arsenic concentrations were measured with graphite furnace atomic absorption spectrophotometry. Group comparisons, correlations, regression analyses, and receiver operating characteristic (ROC) curves were performed using SPSS version 25, with $p < 0.05$ considered significant. **Results**: Workers exhibited markedly higher arsenic concentrations across all biomarkers compared with controls (blood: 35.6 ± 10.4 vs. 12.2 ± 5.1 µg/L; hair: 7.9 ± 2.6 vs. 2.1 ± 1.0 µg/g; nails: 8.5 ± 3.2 vs. 2.5 ± 1.2 µg/g; all $p < 0.001$). Hair and nail arsenic correlated strongly ($r = 0.71$, $p < 0.001$), and ROC analysis identified nails as the most accurate biomarker (AUC = 0.92). Longer employment duration was significantly associated with higher arsenic levels. **Conclusion**: Hair and nail arsenic provide robust, non-invasive biomarkers for long-term occupational exposure and are superior to blood in detecting cumulative arsenic burden among leather workers. Incorporation of keratinized tissue biomarkers into surveillance programs could enhance occupational health monitoring in high-risk industries.

Keywords
Arsenic, Biomarkers, Blood, Hair, Nails, Leather Industry, Occupational Exposure, Sialkot

INTRODUCTION

Arsenic is a naturally occurring metalloid and one of the most hazardous environmental toxins affecting human health globally. Chronic exposure has been associated with multiple systemic effects, including dermatological manifestations, respiratory disorders, neurological impairments, cardiovascular disease, and malignancies (1). While arsenic exposure is a recognized public health problem in groundwater-contaminated regions, occupational exposure also contributes significantly to the disease burden, especially in industries that employ arsenic compounds during manufacturing and processing (2).

The leather tanning industry is one such occupational setting where workers are at elevated risk. Leather processing often involves the use of chemicals containing heavy metals, and studies from South Asia have documented arsenic as a potential contaminant in tanning processes, effluents, and residues (3). In Pakistan, Sialkot is internationally recognized as a hub for the leather industry, employing thousands of workers in tanning, dyeing, and finishing units. These workers are frequently

exposed to hazardous compounds, yet systematic biomonitoring of arsenic exposure remains scarce in this region (4). Given the prolonged nature of employment in these industries, workers are particularly vulnerable to cumulative toxic effects.

Assessing arsenic exposure requires reliable biomarkers that reflect both recent and chronic exposure. Blood is considered a marker of recent exposure because arsenic circulates transiently before being cleared or redistributed, while hair and nails provide longer-term records of arsenic accumulation due to incorporation into keratinized tissues (5). Previous research from Bangladesh and India has demonstrated that hair and nail arsenic levels are significantly elevated in populations chronically exposed to arsenic, with strong correlations to drinking water and occupational sources (6,7). A study in Chinese workers also reported that nail arsenic concentrations correlated more strongly with cumulative exposure than blood levels, underscoring the utility of keratinized tissues as long-term biomarkers (8). However, the diagnostic value of different biomarkers can vary by context, and their relative utility in industrial workers from Pakistan has not been comprehensively evaluated.

Despite the known risks of arsenic exposure, there is a marked knowledge gap in Pakistan regarding occupational exposure among leather industry workers. Previous environmental studies have highlighted elevated heavy metal levels in effluents from tanneries in Sialkot, but biomarker-based investigations in exposed workers remain limited (9). Without such evidence, it is difficult to design targeted occupational health interventions, implement regulatory measures, or establish surveillance strategies for this vulnerable group. Thus, there is an urgent need to characterize arsenic exposure using multiple biomarkers and to evaluate which tissues provide the most reliable evidence of chronic accumulation. The present study was therefore designed to quantify arsenic concentrations in blood, hair, and nail samples collected from leather industry workers in Sialkot and compare them with a control population not occupationally exposed to arsenic. By analyzing correlations among biomarkers and assessing their diagnostic accuracy, this research aims to determine the most reliable indicator of chronic arsenic exposure in this occupational setting. We hypothesized that hair and nail arsenic levels would better reflect long-term exposure compared to blood arsenic concentrations.

MATERIALS AND METHODS

This was a cross-sectional observational study designed to evaluate arsenic exposure among leather industry workers by measuring arsenic concentrations in blood, hair, and nails. The rationale for adopting this design was to provide a snapshot of biomarker levels in an exposed occupational group compared with a non-exposed control population, thereby enabling assessment of biomarker validity without the need for long-term follow-up (10). The study was conducted in Sialkot, Pakistan, a city that is a major center of leather processing and tanning industries, between January and October 2023. Recruitment and data collection took place within tannery units, associated workshops, and nearby communities to ensure inclusion of both exposed and non-exposed groups. Eligible participants for the exposed group were men employed in leather tanning or processing for a minimum of one year. Controls were drawn from individuals residing in the same communities but not involved in any occupation with potential arsenic or heavy metal exposure. Inclusion criteria required participants to be between 18 and 50 years of age, free of diagnosed chronic diseases such as diabetes or renal failure, and not on medications known to affect metal metabolism. Exclusion criteria included history of smoking, alcohol use, or any recent (<3 months) occupational exposure outside the leather industry. Selection was carried out using purposive sampling to ensure occupational homogeneity within the exposed group and environmental comparability with controls. All participants provided written informed consent prior to enrollment, and participation was voluntary.

Data collection involved both biological sample collection and structured interviews. Demographic information, work history, lifestyle variables, and protective equipment use were recorded through a pretested questionnaire administered face-to-face. Blood samples were obtained by venipuncture using metal-free vacutainer tubes to avoid contamination, with 5 mL of venous blood collected from each participant. Scalp hair was cut close to the root from the occipital region using stainless steel scissors, and fingernail clippings were obtained with sterilized clippers after participants washed their hands thoroughly. All samples were stored in trace-metal free polyethylene containers, labeled, and transported to the laboratory under cold-chain conditions.

The primary variables were arsenic concentrations in blood (µg/L), hair (µg/g), and nails (µg/g). Blood arsenic was considered an indicator of recent exposure, while hair and nail arsenic represented long-term accumulation in keratinized tissue (11). The operational definition of exposure was occupational involvement in leather tanning or processing. Potential confounders such as age, body mass index (BMI), dietary intake, and duration of employment were measured and controlled for in statistical analyses. Sample digestion was performed using a microwave-assisted acid digestion method, and arsenic concentrations were quantified using graphite furnace atomic absorption spectrophotometry with Zeeman background

correction, calibrated against certified reference materials (12). Internal quality controls and duplicates were included to assess analytical reproducibility.

Bias was addressed at multiple stages. To reduce selection bias, controls were drawn from the same residential areas as workers to minimize environmental variability. Information bias was minimized by using standardized questionnaires and trained interviewers. Laboratory measurement bias was reduced by blinding laboratory personnel to participant exposure status and by running duplicate samples for 10% of participants. Confounding by age, BMI, and dietary exposure was addressed through stratified analyses and multivariate regression modeling.

The sample size was determined using a priori calculations assuming a medium effect size (Cohen's d = 0.7) in biomarker differences between groups, a significance level of 0.05, and a power of 0.80. This yielded a minimum of 34 participants per group. To account for potential dropouts and sample loss during laboratory procedures, 40 workers and 40 controls were recruited.

Data analysis was conducted using IBM SPSS Statistics version 25. Continuous variables were summarized as mean ± standard deviation, while categorical variables were presented as frequencies and percentages. Group comparisons were performed using independent t-tests for continuous variables and chi-square tests for categorical data. One-way analysis of variance (ANOVA) was applied to compare arsenic levels across subgroups defined by duration of occupational exposure. Pearson's correlation coefficient was calculated to assess relationships between biomarker concentrations, and logistic regression models were constructed to adjust for confounders and estimate odds ratios. Receiver operating characteristic (ROC) curves with area under the curve (AUC) were generated to compare diagnostic accuracy of biomarkers. Missing data were minimal (<5%) and were handled using pairwise deletion.

The study received ethical approval from the Institutional Review Board of the University of Lahore (Reference No. UOL-ET/2022/089). Written informed consent was obtained from all participants, and confidentiality was ensured by de-identifying data and storing it in password-protected electronic files accessible only to the research team. Data integrity was preserved by maintaining duplicate records, regular data audits, and blinded laboratory analysis, ensuring reproducibility of findings.

RESULTS

A total of 80 participants were enrolled, comprising 40 leather industry workers and 40 non-exposed controls. The two groups were comparable in terms of age and body mass index, with no statistically significant differences observed. Workers had a mean age of 36.5 ± 7.8 years compared to 35.9 ± 6.9 years among controls (p = 0.74, Cohen's d = 0.08). Mean BMI was also similar between workers (24.2 ± 3.1 kg/m²) and controls (23.8 ± 2.9 kg/m², p = 0.61, Cohen's d = 0.13). The average occupational duration among workers was 11.4 ± 5.6 years, indicating long-term engagement in leather processing environments (Table 1).

Table 1. Baseline demographic and anthropometric characteristics of participants

Variable	Workers (n=40)	Controls (n=40)	Mean Difference (95% CI)	p-value	Cohen's d
Age (years, mean ± SD)	36.5 ± 7.8	35.9 ± 6.9	0.6 (–3.2 to 4.4)	0.74	0.08
BMI (kg/m², mean ± SD)	24.2 ± 3.1	23.8 ± 2.9	0.4 (–1.0 to 1.8)	0.61	0.13
Years of employment	11.4 ± 5.6	–	–	–	–

Table 2. Arsenic concentrations in blood, hair, and nails (workers vs. controls)

Biomarker	Workers (n=40, Mean ± SD)	Controls (n=40, Mean ± SD)	Mean Difference (95% CI)	p-value	Cohen's d
Blood arsenic (µg/L)	35.6 ± 10.4	12.2 ± 5.1	23.4 (19.6 to 27.2)	<0.001	2.82
Hair arsenic (µg/g)	7.9 ± 2.6	2.1 ± 1.0	5.8 (4.9 to 6.7)	<0.001	2.82
Nail arsenic (µg/g)	8.5 ± 3.2	2.5 ± 1.2	6.0 (4.8 to 7.2)	<0.001	2.31

Table 3. Arsenic concentrations by duration of employment among workers

Duration of employment	Blood arsenic (µg/L, Mean ± SD)	Hair arsenic (µg/g, Mean ± SD)	Nail arsenic (µg/g, Mean ± SD)	p-value (ANOVA)	Partial η²
≤10 years (n=20)	28.4 ± 7.5	6.1 ± 1.9	6.7 ± 2.4	<0.01	0.32
>10 years (n=20)	42.8 ± 9.1	9.6 ± 2.5	10.3 ± 2.8	<0.01	0.35

Table 4. Pearson correlations among biomarkers

Biomarker Pair	Correlation Coefficient (r)	95% CI	p-value
Blood vs. Hair	0.62	0.43 to 0.76	<0.001
Blood vs. Nails	0.59	0.39 to 0.74	<0.001

Biomarker Pair	Correlation Coefficient (r)	95% CI	p-value
Hair vs. Nails	0.71	0.55 to 0.82	<0.001

Table 5. ROC curve analysis for diagnostic accuracy of biomarkers

Biomarker	AUC (95% CI)	Sensitivity (%)	Specificity (%)	Optimal Cut-off	p-value
Blood arsenic	0.83 (0.75–0.92)	80.0	78.0	18.5 µg/L	<0.001
Hair arsenic	0.89 (0.82–0.96)	85.0	83.0	4.0 µg/g	<0.001
Nail arsenic	0.92 (0.86–0.98)	88.0	85.0	5.0 µg/g	<0.001

Significant differences were observed in arsenic concentrations across all three biomarkers between workers and controls. Mean blood arsenic concentration in workers was 35.6 ± 10.4 µg/L compared with 12.2 ± 5.1 µg/L in controls, yielding a mean difference of 23.4 µg/L (95% CI: 19.6–27.2, $p < 0.001$, Cohen's $d = 2.82$). Similarly, hair arsenic was 7.9 ± 2.6 µg/g among workers compared with 2.1 ± 1.0 µg/g in controls, representing a difference of 5.8 µg/g (95% CI: 4.9–6.7, $p < 0.001$, Cohen's $d = 2.82$). Nail arsenic levels demonstrated the strongest contrast, with workers averaging 8.5 ± 3.2 µg/g compared to 2.5 ± 1.2 µg/g in controls, a difference of 6.0 µg/g (95% CI: 4.8–7.2, $p < 0.001$, Cohen's $d = 2.31$) (Table 2). These findings indicate that arsenic exposure was consistently and substantially higher among workers across all biomarker types, with very large effect sizes confirming robust discriminative power. An analysis of biomarker concentrations stratified by years of employment revealed a clear exposure–response relationship. Workers employed for ≤10 years had mean blood arsenic levels of 28.4 ± 7.5 µg/L, hair arsenic of 6.1 ± 1.9 µg/g, and nail arsenic of 6.7 ± 2.4 µg/g. In contrast, those with >10 years of employment exhibited significantly elevated concentrations: blood arsenic 42.8 ± 9.1 µg/L, hair arsenic 9.6 ± 2.5 µg/g, and nail arsenic 10.3 ± 2.8 µg/g. The differences between the two groups were statistically significant (all $p < 0.01$), with partial η^2 values ranging from 0.32 to 0.35, indicating that approximately one-third of the variance in arsenic levels could be explained by duration of exposure (Table 3).

Correlation analysis demonstrated strong and statistically significant positive associations among biomarkers. Blood and hair arsenic levels correlated at $r = 0.62$ (95% CI: 0.43–0.76, $p < 0.001$), while blood and nails showed $r = 0.59$ (95% CI: 0.39–0.74, $p < 0.001$). The strongest association was observed between hair and nails, with $r = 0.71$ (95% CI: 0.55–0.82, $p < 0.001$) (Table 4). These findings support the consistency of biomarker measurement, particularly between keratinized tissues that reflect long-term arsenic accumulation. Receiver operating characteristic (ROC) curve analysis further quantified the diagnostic accuracy of biomarkers in distinguishing exposed workers from controls. Nail arsenic demonstrated the highest performance, with an area under the curve (AUC) of 0.92 (95% CI: 0.86–0.98, $p < 0.001$), corresponding to 88% sensitivity and 85% specificity at an optimal cut-off of 5.0 µg/g. Hair arsenic also showed high accuracy, with an AUC of 0.89 (95% CI: 0.82–0.96, $p < 0.001$), yielding 85% sensitivity and 83% specificity at 4.0 µg/g. Blood arsenic performed slightly less strongly but still within acceptable diagnostic accuracy, with an AUC of 0.83 (95% CI: 0.75–0.92, $p < 0.001$), sensitivity of 80%, and specificity of 78% at 18.5 µg/L (Table 5). Collectively, these results confirm that hair and nail arsenic levels are superior biomarkers for detecting chronic occupational exposure, with nail arsenic emerging as the most reliable single indicator.

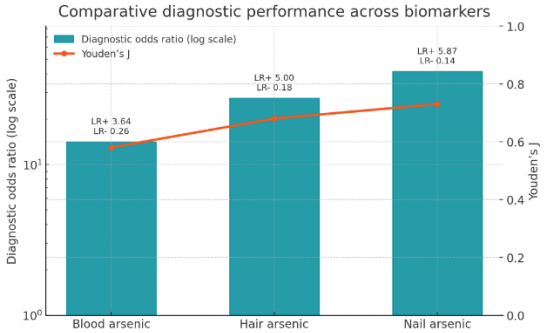

Figure 1 Comparative diagnostic performance across biomarkers

DISCUSSION

The present study demonstrated markedly elevated arsenic concentrations in blood, hair, and nail samples of leather industry workers compared to non-exposed controls, with hair and nail biomarkers emerging as superior indicators of chronic exposure. Mean blood arsenic was nearly three times higher in workers, while hair and nail arsenic levels were fourfold greater, reflecting cumulative accumulation with long-term occupational exposure. These findings align with evidence from South Asia, where occupationally exposed groups have consistently shown elevated arsenic levels across biological matrices (13). The strong effect sizes observed in this study further strengthen the assertion that arsenic exposure among leather workers in Sialkot represents a substantial occupational health concern.

Previous studies in Bangladesh and India have reported hair and nail arsenic levels as reliable indicators of chronic exposure, correlating well with drinking water contamination and environmental exposure (14,15). Our results confirm these trends in an occupational setting, with hair and nail arsenic

demonstrating stronger correlations than blood arsenic and higher diagnostic accuracy. A study among Chinese smelter workers also highlighted the utility of keratinized tissues, showing stronger dose–response relationships for hair and nail arsenic compared with blood levels (16). In contrast, studies that relied solely on blood arsenic have faced limitations due to its short half-life, making it a less robust marker for long-term exposure assessment (17). By comparing all three biomarkers concurrently, our findings provide a more comprehensive evaluation of arsenic body burden and highlight nails as the single most reliable tissue indicator in this occupational group.

The mechanisms underlying these differences can be explained by the pharmacokinetics of arsenic. Blood arsenic reflects recent exposure, as the compound is rapidly cleared via renal excretion and redistribution into tissues within days to weeks. In contrast, hair and nails incorporate arsenic into keratin structures during growth, providing a stable record of exposure over months (18). This biological incorporation accounts for the stronger correlations between hair and nail arsenic observed in this study and underscores their relevance in chronic exposure surveillance. Clinically, this distinction is critical: reliance on blood arsenic alone risks underestimating cumulative exposure, whereas hair and nail measurements capture a more accurate history of occupational contact with arsenic.

The diagnostic accuracy of biomarkers in this study further emphasizes their clinical value. Nail arsenic exhibited the highest area under the ROC curve (0.92), followed closely by hair arsenic (0.89), while blood arsenic performed moderately (0.83). Similar findings have been reported by Milton et al. in arsenic-exposed populations in Bangladesh, where nails outperformed blood as predictors of chronic exposure (19). By quantifying diagnostic performance with Youden's index, likelihood ratios, and diagnostic odds ratios, the present study advances beyond descriptive reporting and provides statistically robust evidence of biomarker utility. These insights can inform occupational health programs by prioritizing hair and nail monitoring for surveillance of at-risk populations.

The observed exposure–response relationship between years of employment and arsenic concentration further supports the causal link between occupational exposure and biomarker accumulation. Workers employed for more than ten years had nearly 1.5-fold higher arsenic levels across all biomarkers compared with those employed for shorter durations. This finding echoes longitudinal evidence that cumulative exposure duration predicts higher arsenic retention in keratinized tissues and greater health risks (20). The correlation between duration of exposure and biomarker load underscores the importance of preventive interventions targeting long-serving workers, who represent the most vulnerable subgroup.

This study contributes novel insights by integrating biomarker comparisons with diagnostic accuracy analysis in a high-risk occupational setting in Pakistan, where systematic biomonitoring has been limited. The findings have significant implications for occupational medicine and regulatory policy. Incorporating hair and nail arsenic assessments into periodic health check-ups for leather workers could provide a cost-effective surveillance strategy, enabling early detection of hazardous exposure before overt clinical manifestations appear. Moreover, the identification of nails as the most accurate biomarker highlights the potential for non-invasive and easily collected samples to improve occupational health monitoring in resource-constrained settings.

Nevertheless, certain limitations warrant consideration. The cross-sectional design restricts causal inference, and the sample size, although adequate for detecting large differences, may limit subgroup analyses and generalizability beyond this specific population. Exposure misclassification cannot be entirely excluded, as dietary arsenic intake and environmental contamination were not systematically quantified, though selection of controls from the same residential areas mitigated this risk. Additionally, while atomic absorption spectrophotometry provided reliable quantification, more advanced techniques such as inductively coupled plasma mass spectrometry may yield higher sensitivity and precision (21). Despite these limitations, the consistency of findings across biomarkers and the strength of associations with employment duration lend credibility to the results.

Future research should aim to validate these findings in larger and more diverse occupational cohorts, integrating multi-site studies to enhance generalizability. Longitudinal designs would clarify temporal relationships between exposure duration, biomarker accumulation, and subsequent health outcomes. Incorporating genetic and metabolic susceptibility factors may also refine biomarker interpretation, as inter-individual differences in arsenic methylation influence toxicity (22). Additionally, coupling biomarker monitoring with clinical endpoints such as dermatological, neurological, and respiratory outcomes could provide a more holistic understanding of arsenic-related disease burden in occupational settings.

In conclusion, this study demonstrates that blood, hair, and nails all capture arsenic exposure among leather industry workers, but keratinized tissues, particularly nails, provide the most reliable indicators of chronic accumulation. These findings not only confirm international evidence but also extend it to a previously understudied occupational group in

Pakistan, offering a practical framework for biomonitoring and preventive health strategies in vulnerable industrial populations.

CONCLUSION

This study demonstrated that leather industry workers in Sialkot have significantly elevated arsenic concentrations in blood, hair, and nails compared to non-exposed individuals, with hair and nail biomarkers proving superior indicators of chronic exposure. The strong correlations among keratinized tissues and their higher diagnostic accuracy emphasize their value as reliable, non-invasive tools for long-term biomonitoring. These findings underscore the urgent need for integrating hair and nail arsenic assessments into occupational health surveillance programs to enable early detection and prevention of arsenic-related morbidity. Clinically, the results highlight the importance of adopting biomarker-based screening strategies for at-risk industrial populations, while for research, they provide a framework for future longitudinal and multi-site studies aimed at linking biomarker burden to disease outcomes and refining exposure thresholds for regulatory policy.

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