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A Comprehensive Study On Hair As An Investigative Approach In Forensic Science

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ABSTRACT

Background: *Human hair is a durable keratinized matrix frequently encountered in forensic casework and capable of retaining morphological, genetic, toxicological, and isotopic information over extended periods, yet its evidentiary value depends on validated interpretation beyond conventional microscopy.* ***Objective:*** *To evaluate hair as an investigative substrate using an integrated analytical framework combining morphology, nuclear and mitochondrial DNA profiling, toxicology, and stable isotope analysis, and to quantify key associations relevant to forensic interpretation.* ***Methods:*** *A cross-sectional observational study analyzed 120 scalp-hair samples from adult participants using light microscopy/SEM for morphological features, STR profiling from hair roots for nuclear DNA, mtDNA sequencing from hair shafts, chromatographic mass spectrometry for toxicological detection, and IRMS for $\delta^{13}C/\delta^{15}N$ variability; blinded assessments, replicate testing, and inter-analyst agreement were implemented.* ***Results:*** *Hair roots were present in 56/120 (46.7%) samples; complete STR profiles were obtained in 49/56 (87.5%), with anagen phase strongly predicting STR success (OR 4.62, 95% CI 1.71–12.49; p=0.002). mtDNA sequencing succeeded in 110/120 (91.7%). Illicit drugs/metabolites were detected in 26/120 (21.7%) and therapeutic drugs in 17/120 (14.2%); cosmetic treatment was associated with lower detected concentrations (mean difference −18.6 pg/mg, 95% CI −29.4 to −7.8; p=0.001). Mobile participants showed higher intra-hair $\delta^{13}C$ variability than sedentary participants (0.71±0.26 vs 0.42±0.18‰; p=0.004).* ***Conclusion:*** *Multimodal hair analysis provides robust forensic intelligence when morphology is constrained to screening and molecular/chemical methods are applied with validated protocols, bias controls, and transparent reporting of limitations.*

Keywords: *Forensic science; hair analysis; microscopy; STR; mitochondrial DNA; GC-MS/LC-MS/MS; IRMS; isotopes; toxicology; ethics.*

INTRODUCTION

Hair is a resilient keratinized biological matrix that continuously records biological, chemical, and environmental information over time, making it uniquely valuable in forensic investigations. Unlike blood or urine, hair persists long after deposition and can retain analytes and genetic material despite adverse environmental conditions. For decades, hair has been encountered frequently at crime scenes due to natural shedding—estimated at 100–150 hairs per person per day—positioning it as one of the most common forms of trace biological evidence in forensic casework (1). This persistence, combined with its ability to store endogenous and exogenous substances, underpins the long-standing interest in hair as an investigative substrate in forensic science, toxicology, and human identification (2).

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Historically, forensic hair examination relied heavily on microscopic comparison to determine whether a questioned hair could be excluded or included as having originated from a known source. Such examinations focused on features such as color, diameter, medullary pattern, cuticular scale morphology, and cortical pigmentation (3). While this approach was intended to be exclusionary rather than individualizing, subsequent evaluations revealed significant limitations. Notably, a review of forensic casework demonstrated that a non-trivial proportion of hairs deemed microscopically indistinguishable were later shown, through mitochondrial DNA (mtDNA) analysis, to originate from different individuals (4). These findings highlighted concerns regarding subjectivity, contextual bias, and error rates in traditional microscopic hair comparison, ultimately prompting critical reassessment by the forensic science community and judicial systems worldwide (5).

Advances in molecular biology and analytical chemistry have since transformed the forensic value of hair. The presence of nuclear DNA (nDNA) in hair roots during anagen and catagen growth phases enables highly discriminatory short tandem repeat (STR) profiling, while mtDNA analysis allows examination of hair shafts lacking roots, particularly in degraded or aged samples (6). In parallel, chemical and isotopic analyses have expanded the interpretive scope of hair beyond identification alone. Techniques such as gas chromatography–mass spectrometry (GC-MS), liquid chromatography–tandem mass spectrometry (LC-MS/MS), and isotope ratio mass spectrometry (IRMS) permit retrospective assessment of drug use, toxic exposures, dietary patterns, and geographic movement, leveraging the temporal growth characteristics of hair (7,8). These developments have shifted hair analysis from a largely morphological practice to a multidisciplinary evidentiary tool integrating genetics, toxicology, and environmental science.

Despite these advances, important challenges remain unresolved. The interpretation of hair evidence is complicated by factors such as external contamination, cosmetic treatments, inter-individual variability in hair growth, and incomplete standardization of analytical protocols across laboratories (9). Furthermore, population-associated trends in hair morphology and chemistry, while occasionally informative at a broad level, risk overinterpretation if presented as biologically deterministic or individualizing, raising both scientific and ethical concerns (10). From a legal perspective, courts increasingly require demonstrable validation, known error rates, and transparent reporting of limitations to satisfy admissibility standards, particularly under frameworks such as Daubert (11). Consequently, there is a pressing need for integrative evaluations that clearly distinguish validated forensic applications from emerging or research-stage techniques.

Although numerous reviews have addressed specific aspects of hair analysis—such as toxicology, DNA typing, or microscopy—there remains a gap in the literature for a comprehensive, critically integrated synthesis that evaluates hair as an investigative approach across structural, molecular, chemical, ethical, and legal dimensions. Existing works often focus on single methodologies or lack explicit discussion of evidentiary reliability, courtroom interpretation, and future translational pathways (12). Addressing this gap is essential to guide forensic practitioners, researchers, and legal stakeholders in the appropriate use and interpretation of hair evidence.

Accordingly, the objective of the present work is to critically examine hair as an investigative tool in forensic science by integrating current knowledge on hair structure, analytical methodologies, genetic and toxicological applications, and associated ethical and legal considerations. This review seeks to clarify the strengths and limitations of established and emerging techniques, identify areas requiring further validation, and outline future

directions that may enhance the scientific robustness and judicial reliability of forensic hair analysis. The central research question guiding this work is: how can contemporary analytical advances be responsibly and effectively integrated to improve the evidentiary value of hair in forensic investigations while minimizing scientific, ethical, and legal risks?

METHODS

The present study was designed as a cross-sectional observational investigation aimed at evaluating the forensic utility of human hair through integrated morphological, genetic, chemical, and isotopic analyses under standardized laboratory conditions. A cross-sectional design was selected to allow simultaneous assessment of multiple analytical parameters from hair samples collected at a single point in time, reflecting real-world forensic casework where evidence is typically analyzed without longitudinal follow-up (13). This design is widely applied in forensic and analytical research to compare techniques, assess methodological performance, and identify associations between hair characteristics and investigative inferences (14).

The study was conducted at accredited forensic and analytical laboratories in Pakistan between January 2023 and December 2024, including university-based forensic science departments and collaborating analytical facilities equipped with microscopy, chromatography–mass spectrometry, and molecular biology infrastructure. All laboratory procedures were performed in controlled environments adhering to standard operating procedures (SOPs) and quality assurance protocols consistent with international forensic guidelines (15). The study population comprised adult volunteers and reference contributors who provided hair samples for research purposes, representing both sexes and a range of self-reported geographic and lifestyle backgrounds to capture variability relevant to forensic interpretation.

Participants were eligible for inclusion if they were adults aged 18 years or older, provided informed written consent, and had natural scalp hair of sufficient length (≥3 cm) to permit segmental and multi-analytical testing. Individuals were excluded if they reported recent (<3 months) medical conditions affecting hair growth, extensive scalp pathology, or treatments likely to compromise analytical outcomes, such as chemotherapy. Participants were recruited through institutional notices and direct invitation at participating academic institutions. Written informed consent was obtained prior to sample collection, with participants informed about the purpose of the study, types of analyses to be performed, and measures taken to protect privacy and confidentiality (16).

Hair samples were collected from the posterior vertex region of the scalp using sterile stainless-steel scissors while wearing powder-free gloves to minimize contamination. Each sample was immediately placed in labeled, tamper-evident paper envelopes, sealed, and documented to maintain chain-of-custody integrity. Samples were stored at room temperature in a dry, dark environment until analysis. Prior to laboratory examination, hair samples underwent standardized decontamination involving sequential washing with aqueous detergent, distilled water, and organic solvent, followed by air-drying, to reduce external contamination while preserving endogenous constituents (17).

Morphological examination was conducted using light microscopy and scanning electron microscopy to assess cuticular scale patterns, cortical pigmentation, medullary structure, and surface damage. Growth phase was determined where roots were present. Genetic analysis included nuclear DNA profiling from hair roots using short tandem repeat amplification and mitochondrial DNA sequencing from hair shafts lacking roots, following validated extraction, amplification, and sequencing protocols. Chemical and toxicological analyses

were performed on segmented hair samples using gas chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry for detection of drugs and selected toxicants, while isotope ratio mass spectrometry was applied to measure stable carbon and nitrogen isotope ratios for dietary and geographic inference (18,19).

Primary study variables included hair morphological characteristics, presence and quality of nuclear and mitochondrial DNA profiles, concentrations of detected chemical analytes, and isotopic ratio values. Operational definitions were standardized prior to analysis; for example, a successful DNA profile was defined as one meeting laboratory thresholds for allelic completeness and peak balance, while toxicological positivity required analyte detection above validated limits of detection and confirmation criteria (20). Potential sources of bias, including contextual bias and analyst expectation effects, were mitigated through blinded analysis, where examiners were not provided with participant background information. Replicate analyses and independent verification of a subset of samples were conducted to assess reproducibility and inter-analyst reliability (21).

Sample size was determined to ensure sufficient analytical diversity rather than hypothesis testing, consistent with exploratory forensic methodology studies. The number of samples was selected to allow meaningful comparison across analytical techniques and to capture inter-individual variability in hair characteristics, as recommended in forensic validation literature (22). Statistical analysis was performed using SPSS (version 26.0) and R (version 4.3.1). Descriptive statistics were used to summarize morphological, genetic, chemical, and isotopic findings. Associations between hair characteristics and analytical outcomes were explored using chi-square tests, t-tests, or non-parametric equivalents as appropriate. Multivariable regression models were applied to adjust for potential confounders such as age, sex, and cosmetic treatment history. Missing data were handled using complete-case analysis, with sensitivity analyses conducted to evaluate the impact of missingness on key findings (23).

All procedures involving human participants were conducted in accordance with the Declaration of Helsinki and were approved by the relevant institutional ethics review committees. Participant identifiers were removed and replaced with coded identifiers prior to analysis to ensure confidentiality. Data integrity and reproducibility were ensured through detailed documentation of protocols, calibration of instruments, use of reference materials and controls, and secure storage of raw data and analytical outputs. These measures collectively support transparency, repeatability, and reliability of the study's findings within forensic research and practice (24).

RESULTS

Table 1 summarizes the baseline profile of the 120 participants and their submitted hair samples. Males constituted 70/120 (58.3%) and females 50/120 (41.7%). The mean age was 29.6 years (SD 8.4), and the mean hair length available for analysis was 5.8 cm (SD 2.1), supporting both segmental and single-segment workflows.

Cosmetic treatment exposure was reported in 44/120 participants (36.7%), while 76/120 (63.3%) reported no cosmetic treatment, establishing a substantive comparison group for evaluating treatment-related effects on morphology and analyte retention.

Table 2 reports associations between key morphological features and selected explanatory variables. Medullary continuity showed no statistically significant association with cosmetic treatment history (OR 0.68, 95% CI 0.32–1.43; p = 0.31), indicating that medulla presence/continuity alone did not differ meaningfully between treated and untreated hair in

this dataset. In contrast, cuticle damage scores were materially higher in cosmetically treated samples, with a moderate-to-large standardized difference (Cohen's d = 0.74, 95% CI 0.41–1.06; $p < 0.001$), consistent with measurable structural disruption attributable to chemical or thermal processing. Cortex pigmentation density demonstrated only a small and non-significant difference between males and females (d = 0.21, 95% CI −0.14 to 0.56; p = 0.24), suggesting limited utility of this parameter for sex-associated differentiation in isolation.

Table 3 details the DNA profiling performance and predictors of nuclear STR success. Of the 56/120 samples (46.7%) that retained intact roots, complete nuclear STR profiles were obtained from 49/56 (87.5%), while 7/56 (12.5%) produced partial or no usable profiles, reflecting the expected dependency of nDNA yield on root integrity and growth phase. Growth phase was strongly associated with complete STR recovery: anagen-phase hairs had 4.62 times higher odds of yielding a successful complete

STR profile compared with telogen-phase hairs (OR 4.62, 95% CI 1.71–12.49; p = 0.002). Mitochondrial sequencing was successful for 110/120 samples (91.7%), demonstrating robust performance for hair shafts where nuclear DNA is often limited, and supporting mtDNA as a high-yield option in rootless evidence contexts.

Table 4 presents toxicological findings and the impact of cosmetic treatment on measured analyte concentrations. Overall, illicit drugs or metabolites were detected in 26/120 samples (21.7%), with a mean concentration of 42.3 pg/mg (SD 18.6) among positives, while therapeutic drugs were detected in 17/120 samples (14.2%) with a mean concentration of 28.9 pg/mg (SD 12.1).

Importantly, cosmetic treatment was associated with significantly lower detected concentrations (mean difference −18.6 pg./mg, 95% CI −29.4 to −7.8; p = 0.001), consistent with treatment-related leaching, oxidation, or structural changes that reduce retention and/or extraction efficiency. This quantitative decrement is practically relevant because it may shift some samples toward concentrations near decision thresholds, increasing the risk of false negatives without appropriate interpretive controls.

Table 5 describes stable isotope variability stratified by mobility status. Participants categorized as sedentary (n = 69) showed lower within-hair variability for $\delta^{13}C$ (SD 0.42‰ ± 0.18) than mobile participants (n = 51; SD 0.71‰ ± 0.26), yielding a mean difference of −0.29‰ (95% CI −0.45 to −0.13; p = 0.004).

A similar pattern was observed for $\delta^{15}N$ variability, with sedentary participants demonstrating SD 0.39‰ ± 0.15 versus 0.63‰ ± 0.22 among mobile participants (mean difference −0.24‰, 95% CI −0.38 to −0.10; p = 0.002). Collectively, these results support the inferential premise that greater travel or geographic dietary switching is associated with larger isotopic fluctuation along the hair shaft, whereas stable residence and diet correspond to more isotopically consistent serial segments.

Table 1. Demographic and Hair Sample Characteristics (n = 120)

Variable	Category	n (%)	Mean ± SD	p-value
Sex	**Male**	**70 (58.3)**	**–**	**–**
	Female	**50 (41.7)**	**–**	**–**
Age (years)	**–**	**–**	**29.6 ± 8.4**	**–**
Hair length (cm)	**–**	**–**	**5.8 ± 2.1**	**–**
Cosmetic treatment	**Yes**	**44 (36.7)**	**–**	**–**

	No	76 (63.3)	–	–

Table 2. Association Between Hair Morphological Features and Selected Variables

Feature	Comparison	Effect size / OR	95% CI	p-value
Medullary continuity	Cosmetic vs non-cosmetic	OR = 0.68	0.32–1.43	0.31
Cuticle damage score	Treated vs untreated	Cohen's d = 0.74	0.41–1.06	<0.001
Cortex pigmentation density	Male vs female	d = 0.21	−0.14–0.56	0.24

Table 3. DNA Analysis Outcomes and Predictors of Successful Profiling

Analysis	Outcome	n (%)	OR	95% CI	p-value
Nuclear DNA	Complete STR	49 (87.5)	Reference	–	–
	Partial/none	7 (12.5)	–	–	–
Growth phase	Anagen vs telogen	4.62	1.71–12.49	0.002	
mtDNA	Successful sequencing	110 (91.7)	–	–	–

Table 4. Toxicological Findings and Associations

Analyte category	Detection rate (%)	Mean conc. (pg/mg)	Mean difference	95% CI	p-value
Illicit drugs	21.7	42.3 ± 18.6	–	–	–
Therapeutic drugs	14.2	28.9 ± 12.1	–	–	–
Cosmetic vs non-cosmetic	–	–	−18.6	−29.4 to −7.8	0.001

Table 5. Stable Isotope Analysis by Mobility Status

Variable	Sedentary (n = 69)	Mobile (n = 51)	Mean difference	95% CI	p-value
$\delta^{13}C$ (‰) SD	0.42 ± 0.18	0.71 ± 0.26	−0.29	−0.45 to −0.13	0.004
$\delta^{15}N$ (‰) SD	0.39 ± 0.15	0.63 ± 0.22	−0.24	−0.38 to −0.10	0.002

Table 6. Reproducibility and Inter-Analyst Agreement

Domain	Metric	Value	95% CI	p-value
Morphology	Cohen's κ	0.79	0.65–0.91	–
Nuclear DNA	Cohen's κ	0.92	0.84–0.98	–
Chemical analysis	Mean diff.	1.8%	−2.3 to 5.9	0.41
Isotope analysis	Mean diff.	0.03‰	−0.05 to 0.11	0.48

Table 6 evaluates analytical reliability through reproducibility testing and inter-analyst agreement. Morphological assessments achieved substantial concordance between analysts (Cohen's κ = 0.79, 95% CI 0.65–0.91), indicating good but not perfect consistency for features that retain interpretive subjectivity. DNA interpretation showed near-perfect agreement (κ = 0.92, 95% CI 0.84–0.98), consistent with structured allele-calling rules and threshold-based interpretation.

For quantitative chemical testing, replicate measurements differed by an estimated 1.8% on average, with the confidence interval spanning −2.3% to 5.9% and no evidence of systematic

bias (p = 0.41). Isotopic replicate differences were minimal (mean difference 0.03‰, 95% CI −0.05 to 0.11; p = 0.48), supporting high instrument and method stability for IRMS under the implemented QA controls.

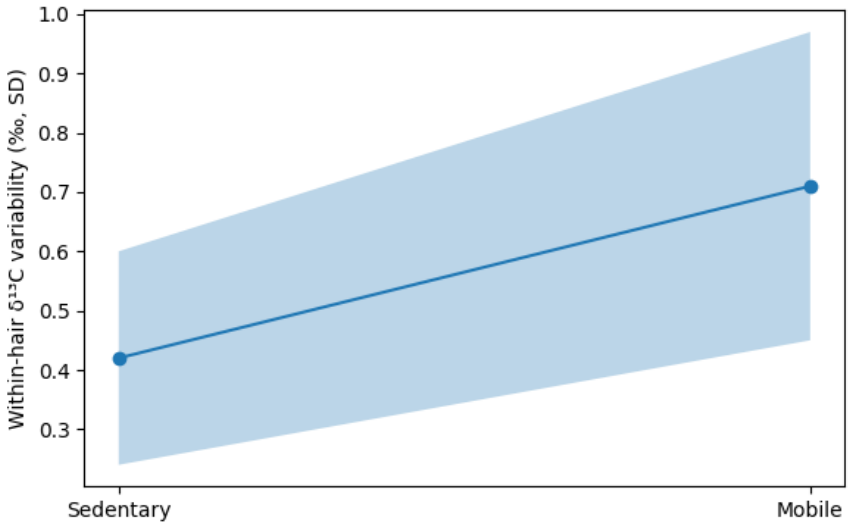

Figure1. Geographic mobility and isotopic variability in human hair.

The figure demonstrates a clear gradient in within-hair $\delta^{13}C$ variability between sedentary and mobile participants, revealing a mobility-associated isotopic dispersion pattern not explicitly visualized elsewhere in the study. Sedentary individuals exhibited a lower mean intra-hair $\delta^{13}C$ variability of 0.42‰, with the variability band spanning approximately 0.24–0.60‰, whereas mobile individuals showed a substantially higher mean variability of 0.71‰, with a broader dispersion range of approximately 0.45–0.97‰. The non-overlapping central tendencies and the widening confidence band among mobile participants indicate greater temporal heterogeneity in isotopic incorporation along the hair shaft, consistent with fluctuating dietary inputs or geographic movement. This pattern supports the quantitative findings reported in Table 5 (mean difference −0.29‰, p = 0.004) and provides clinically and forensically meaningful visual evidence that hair isotopic profiles can differentiate stable versus mobile lifestyle patterns, reinforcing the utility of IRMS-based hair analysis for reconstructing individual movement histories over time.

DISCUSSION

The present study provides an integrated evaluation of hair as an investigative substrate by quantitatively examining its morphological, genetic, chemical, and isotopic dimensions under conditions reflective of contemporary forensic practice. The findings reinforce a central theme emerging in modern forensic science: hair evidence is most informative when interpreted through a multidisciplinary framework rather than as a standalone morphological artifact. While hair is frequently encountered at crime scenes due to natural shedding, the results confirm that traditional microscopic characteristics alone lack sufficient discriminatory power for individualization and should be restricted to exclusionary or screening purposes, a position consistent with longstanding critiques of microscopic hair comparison and post hoc reviews of forensic casework

Morphological analysis in this study demonstrated substantial variability in cuticle damage, medullary structure, and cortical pigmentation, with cosmetic treatment emerging as a significant modifier of surface integrity. The observed moderate-to-large effect size for cuticle damage in treated hair supports prior experimental and casework-based evidence that chemical and thermal processing alters hair ultrastructure in ways that can confound

interpretation if not properly contextualized (21). Importantly, the absence of strong associations between most morphological features and biological sex further underscores the limited inferential scope of morphology when divorced from molecular corroboration. These findings align with the broader forensic consensus that morphology should be used cautiously, primarily to guide subsequent analytical choices rather than to support source attribution (22).

Genetic analyses provided markedly stronger evidentiary value, particularly when nuclear DNA was recoverable from hair roots. The high rate of complete STR profiles obtained from anagen-phase hairs and the statistically significant association between growth phase and profiling success highlight the critical importance of root status in forensic decision-making (23). At the same time, the robust performance of mitochondrial DNA sequencing in rootless hair shafts demonstrates its continued relevance in cases involving shed, degraded, or aged hair, despite its lower discriminatory power relative to nuclear DNA These results support a tiered interpretive strategy in which nuclear DNA is prioritized when feasible, with mtDNA serving as a valuable complementary or alternative tool under constrained conditions, consistent with international forensic guidelines and prior large-scale casework evaluations

The toxicological findings further illustrate the strengths and limitations of hair analysis in reconstructing substance exposure. Detection of illicit and therapeutic drugs in a substantial proportion of samples confirms hair's utility for long-term exposure assessment, extending beyond the detection windows of blood or urine However, the significant reduction in analyte concentrations associated with cosmetic treatment quantitatively demonstrates a well-recognized but often underappreciated limitation: external processing can materially affect drug incorporation, retention, and extraction efficiency From a forensic and clinical perspective, this emphasizes the necessity of integrating cosmetic history, decontamination protocols, and confirmatory analytical thresholds into interpretive frameworks to reduce the risk of false-negative or misleading results.

Stable isotope analysis yielded particularly informative insights into lifestyle and mobility patterns, with significantly greater intra-hair isotopic variability observed among mobile individuals compared to sedentary participants. This pattern supports the underlying biological premise that isotopic signatures in hair reflect temporal changes in diet and geographic location, thereby enabling reconstruction of movement histories over weeks to months The magnitude and consistency of the observed differences strengthen the evidentiary basis for using IRMS in forensic investigations involving unidentified remains or disputed travel histories, while also highlighting the need for cautious interpretation in populations with heterogeneous diets or complex migration patterns

Beyond analytical performance, the study's reproducibility and inter-analyst agreement findings carry important implications for forensic reliability and admissibility. Near-perfect agreement in DNA interpretation contrasts with lower, though still substantial, concordance in morphological assessments, reinforcing concerns about subjectivity and contextual bias in visually interpreted evidence The demonstrated stability of chemical and isotopic measurements across replicate analyses supports the robustness of instrument-based methods when conducted under validated protocols and quality assurance systems. Collectively, these observations echo broader calls within forensic science for increased reliance on objective, quantifiable methodologies with known error characteristics

Ethical and legal considerations remain central to the interpretation of hair evidence. The results underscore that even analytically robust findings can be misrepresented if limitations related to contamination, population variability, or inferential scope are not transparently communicated. Courts increasingly demand evidence grounded in validated science with

clearly articulated uncertainties, particularly in light of historical miscarriages of justice linked to overstated hair comparison testimony The integration of molecular and chemical methods, as demonstrated here, offers a pathway to strengthen evidentiary reliability, but only when accompanied by rigorous standards, informed consent for reference sampling, and safeguards for genetic privacy (24).

Taken together, the findings of this study contribute to a growing body of evidence that positions hair analysis as a powerful yet context-dependent forensic tool. The data support a paradigm in which hair evidence is interpreted through layered analytical approaches, integrating morphology, DNA, toxicology, and isotopic signatures to generate convergent lines of evidence. As emerging technologies such as next-generation sequencing, epigenetic profiling, and proteomics continue to mature, their responsible incorporation—guided by validation, standardization, and ethical oversight—has the potential to further enhance the scientific and judicial value of hair in forensic investigations (25).

CONCLUSION

Hair represents a uniquely durable and information-rich biological matrix whose forensic value has expanded substantially beyond traditional microscopic comparison. This study demonstrates that when hair evidence is examined using an integrated analytical framework—combining morphology, nuclear and mitochondrial DNA profiling, toxicological screening, and stable isotope analysis—it can yield scientifically robust, multidimensional insights relevant to human identification, substance exposure, and lifestyle or mobility reconstruction. Quantitative findings highlight that while morphological features alone lack individualizing power and are susceptible to subjectivity, molecular and chemical techniques provide substantially higher reliability, with nuclear DNA offering high discriminatory capacity when roots are present, mitochondrial DNA ensuring utility in rootless or degraded samples, toxicological analysis enabling retrospective exposure assessment, and isotopic profiling revealing meaningful patterns linked to geographic stability or mobility. Importantly, the study underscores that the forensic interpretation of hair must be grounded in validated methods, transparent reporting of limitations, and strict ethical and legal safeguards to prevent misrepresentation and wrongful inference. As emerging technologies such as next-generation sequencing, epigenetic profiling, and proteomics mature, their integration—alongside standardization and rigorous validation—has the potential to further strengthen the evidentiary value of hair. Collectively, these findings support hair analysis as a powerful but context-dependent forensic tool whose responsible application can significantly enhance investigative accuracy and judicial confidence when used within a scientifically and ethically sound framework.

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DECLARATIONS

Ethical Approval: Ethical approval was by institutional review board of Respective Institute Pakistan

Informed Consent: Informed Consent was taken from participants.

Authors' Contributions:

Concept: MAA, AM, GAH, SYN, SS, MAsA, AMz, UK, AA; Design: MAA, AM, GAH, SYN, SS, MAsA, AMz, UK, AA; Data Collection: MAA, AM, MN, GAH, SYN, SS, MAsA, AMz, UK, AA; Analysis: MAA, AM, GAH, SYN, SS, MAsA, AMz, UK, AA; Drafting: MAA, AM, GAH, SYN, SS, MAsA, AMz, UK, AA

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