National Board of Examination - Journal of Medical Sciences
Volume 1, Issue 6, Pages 365–375, June 2023
DOI 10.61770/NBEJMS.2023.v01.i06.006

REVIEW ARTICLE

A Narrative Review on Forensic Toxicology of Human Hair and Nails

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Accepted: 24-May-2023 / Published Online: 01-June-2023

Abstract
Forensic trichology is the study of hair evidence in criminal investigations, helping identify individuals and provide insights into their activities and associations. Forensic onychology, or forensic nail examination, involves analyzing nail evidence to determine factors like cause of death, drug use, or occupational history, assisting in uncovering critical information in forensic investigations. The analytical toxicology of human hair and nails has advanced significantly in recent years, allowing for a more accurate and comprehensive assessment of exposure to drugs, chemicals, and other toxic substances. Hair and nails are increasingly employed as matrices for the detection of new pharmacological targets because of their special qualities, such as their capacity to absorb and store xenobiotics for extended periods of time. However, interpreting the evidence in this area is overly complex and requires strong forensic expertise and adherence to strict scientific protocols. This review will discuss current research in forensic tricho-toxicology and forensic onycho-toxicology.

Keywords: Crime, Forensic Toxicology, Nails, Xenobiotics, Hair Preparations, Poisons

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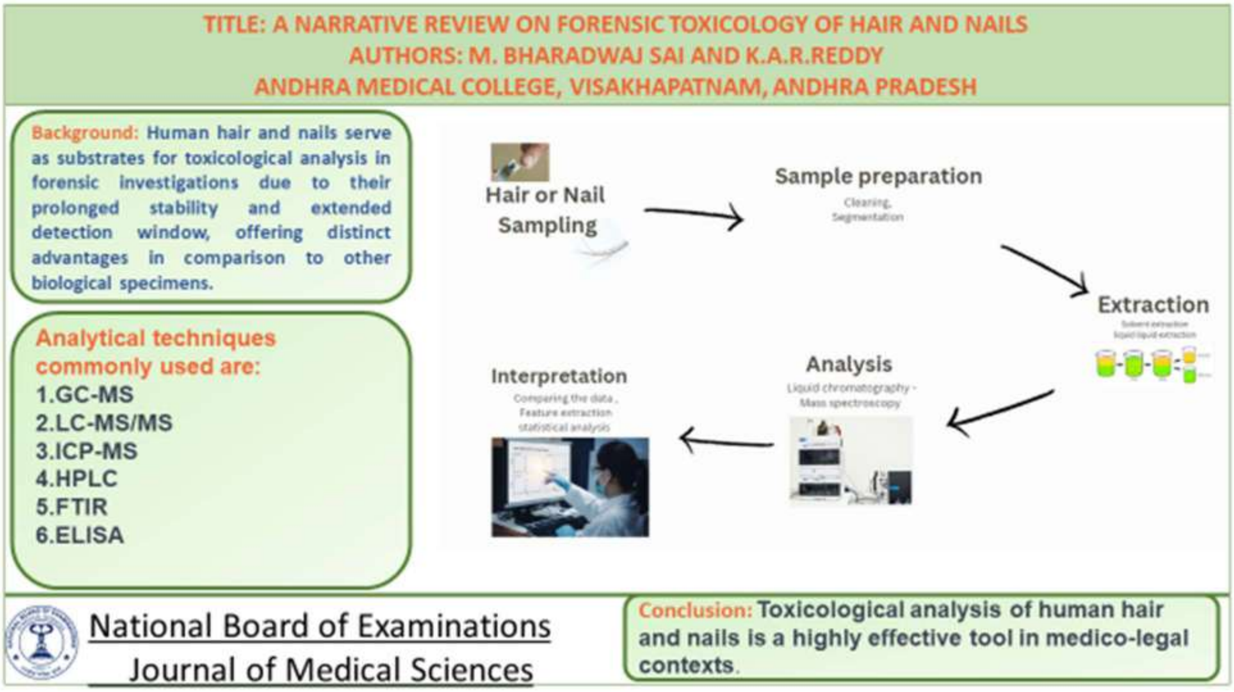
Graphical Abstract



Background

In forensic toxicology, human hair and nails are increasingly being employed as matrices for detecting drugs and toxicants because of their ability to incorporate drugs of abuse for a long duration of time. The history of a person's drug usage or exposure can be inferred retroactively from the hair and nails, which are non-invasive matrices for analysis. In this brief review, we present the most recent studies in the fields of forensic tricho-toxicology and onycho-toxicology along with some future perspectives of research. It is quite uncommon for forensic practitioners to consider hair and nails for toxicological investigations both in the living and the dead. There is a strong need for the dissemination of information on this subject amongst medical legal practitioners [1–3].

Discussion

Hair is a filamentous structure that grows from follicles in the skin. It consists of three layers: the cuticle, cortex, and medulla. The hair follicle beneath the skin contains the papilla, bulb, and root. Nails are composed of the nail plate, nail bed, lunula, and cuticle. The nail plate is the visible part made of tightly packed cells. The nail bed is the skin beneath the nail plate, while the lunula is the crescent-shaped area at the base. The cuticle protects the nail base, and the nail bed supports nail growth. The proximal hair shaft and the nail plate are ideal samples for toxicological analysis (Figure 1).

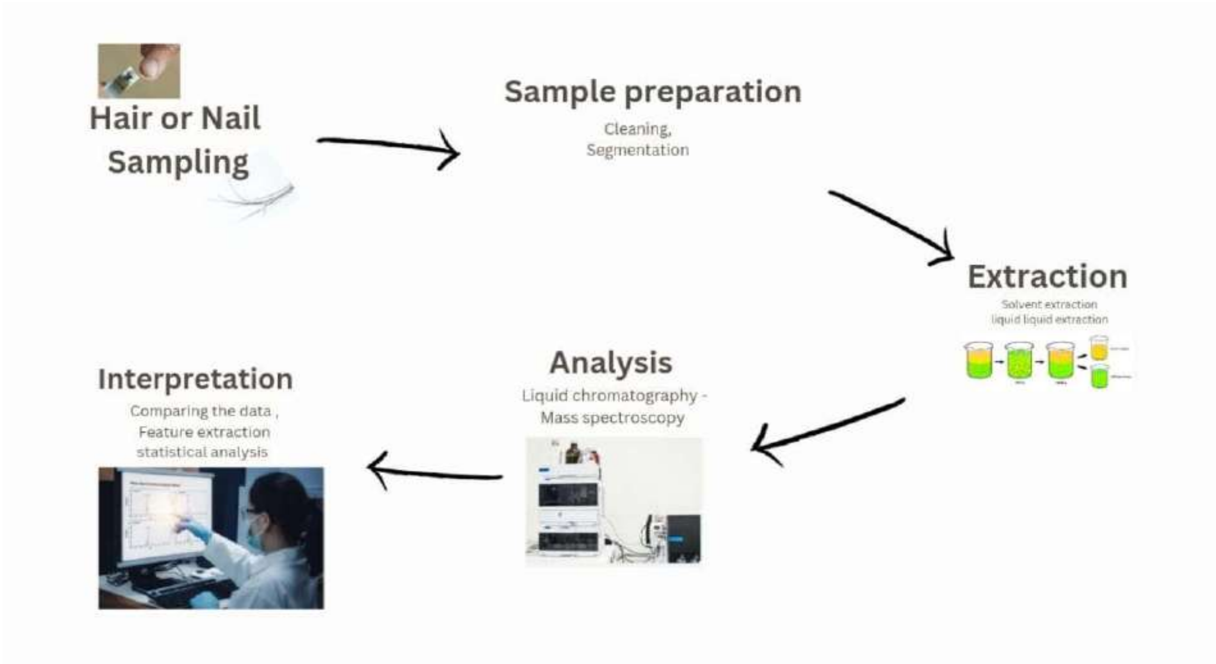


Figure 1. Basic workflow of Toxicological analysis of hair and nails

The collection and processing of hair and nails plays a crucial role in ensuring the accuracy and reliability of analytical toxicology findings. To achieve optimal results in analysis, it is recommended to collect dry samples of hair with roots intact and whole nails. These specimens do not require specific preservatives, although precautions should be taken to prevent fungal contamination. In recent times, advancements in hair and nail sampling and preparation techniques have enabled the examination of lower sample volumes with improved sensitivity and specificity. This progress has been instrumental in enhancing our ability to spatially study drug distribution in hair and nails. Methods such as imaging mass spectrometry and micro-segmental hair analysis have emerged, allowing for the investigation of drug distribution patterns within these matrices. These advancements have contributed to greater precision and a deeper understanding of drug incorporation and deposition in hair and nails [4-7].

One in every five deaths worldwide (11.8 million per year), is reportedly caused directly or indirectly by substance use, according to a statistical report covering the years 1990 to 2019. 1.65 million people lost their lives in India every year due to the drug menace. According to data provided by the World Health Organization, around 270 million people, or 5.5% of the world's population, between the ages of 15 and 64, took psychoactive drugs in the preceding year, and thirty-five million people are thought to be affected by drug use disorders. Drug use or consumption accounts for 75% of all occurrences of predatory rape, which causes unconsciousness and impairs the victim's capacity to consent to sex [8,9].

In forensic toxicology, human hair and nails serve as a good sample to represent chronic exposure to different substances. The history of a person’s drug abuse or exposure can be inferred retrospectively from the hair and nails, which are considered the best non-invasive matrices. Numerous research articles have explored the long inspection window of hair and nails as a means to examine drugs and toxins. In some cases, studies have been conducted on exhumed bodies to detect the presence of drugs or toxins, enabling the determination of the time interval during which these substances were present in the human body [10,11].

The research conducted in the previous decade has focused on various investigative procedures related to different drugs of abuse and their metabolites, utilizing diverse biological samples such as saliva, blood, and hair. Several countries are currently exploring and analyzing alternative samples for drug testing, moving beyond traditional samples like urine or blood. Hair and nail analysis, proven to be effective in the treatment of drug addicts, has also played a crucial role in solving drug-facilitated crimes such as rape cases and child sexual abuse cases.

Hair analysis initially emerged during the 1960s to 1970s for investigating heavy metal poisoning using atomic absorption spectrometry. Subsequent extensive research on hair analysis during the 1970s and 1980s primarily focused on heroin addicts. Presently, the most commonly employed method for analyzing drug abuse in addicts' hair is GC-MS. Many researchers consider hair and nails as optimal sources for drug and toxin analysis due to their longer inspection window compared to other biological fluids from the human body.

The field of toxicology and hair/nail analysis has witnessed significant advancements in instrumentation over time, driven by researchers' pursuit of improved analytical technology and the need for more accurate and sensitive detection methods. Initially, Gas chromatography (GC) was the primary method utilized. In the 1970s and 1980s, GC was coupled with a Flame ionization detector (FID) and widely acclaimed for drug analysis in hair and nails. Subsequently, atomic absorption spectrometry emerged as a technique for hair analysis, particularly in the investigation of heavy metal poisoning. In the 1970s, Gas chromatography – Mass spectrometry (GC-MS) revolutionized the field of analytical toxicology, enabling the identification and quantification of drugs in hair analysis. During the 1980s, Liquid chromatography (LC) came into existence, gaining prominence as High-Performance Liquid Chromatography (HPLC) due to its ability to analyze non-volatile and polar compounds.

Later, in the 1990s and 2000s, LC was combined with mass spectrometry, providing a broader range of compound analyses, including polar and non-polar compounds. As technology continued to evolve, different instrumentation techniques such as Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), Fourier Transform Infrared Spectroscopy (FTIR), and X-ray Fluorescence (XRF) were developed and utilized in the field of toxicology for hair and nail analysis.

Analytical toxicology of human hair has witnessed significant advancements in recent years, enabling more precise and comprehensive assessments of exposure to drugs, chemicals, and other toxic substances. One key area of progress is biomonitoring, where hair and nails have been recognized as valuable matrices for evaluating individuals' exposure to environmental chemicals and contaminants. The use of an atomic absorption spectrometer allows for the detection of heavy metals like lead and cadmium in these samples.

In forensic drug testing, hair and nails are frequently employed to demonstrate long-term drug consumption. Hair is particularly suitable for detecting most basic chemicals, while nails are effective for neutral and weakly acidic substances. Hair and nails also serve as indicators of chronic exposure to environmental toxins, including industrial chemicals, heavy metals, and persistent organic pollutants. An intriguing study on chlorinated paraffin (CPF) exposure revealed that CPF levels increase proportionally to individuals' age. This finding suggests that the accumulation of chlorinated paraffin in hair and nails may primarily depend on a person's age and in turn reflect the duration of exposure.

In the field of forensic toxicology, hair, and nails have emerged as valuable sources of evidence. Analytical techniques have been refined to identify various chemicals in these matrices, including pharmaceuticals, toxins, and poisons. Moreover, isotopic analysis of hair can unveil information about a person's geographic origin and travel history, aiding in forensic geo-profiling. Hair and nails also offer insights into occupational exposure to harmful chemicals. Researchers have investigated the connection between workplace exposure and the accumulation of toxins in these matrices, providing valuable information for assessing occupational health hazards. The advancements in the analytical toxicology of hair and nails have enhanced our ability to assess exposure to drugs, chemicals, and toxins, contributing to various fields such as biomonitoring, forensic science, and occupational health. These developments continue to improve our understanding of human exposure and aid in the detection and prevention of potential health risks [12,13].

The advent of high-resolution mass spectrometry has brought about a revolutionary change in hair analysis, enabling the detection and quantification of a broad range of compounds even at exceptionally low concentrations. Baumgartner et al. (2021) developed an overly sensitive and reliable method using ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) to simultaneously determine opioids, stimulants, benzodiazepines, and new psychoactive substances (NPS) in hair samples. Salomone et al. (2020) provided a comprehensive overview of recent advancements in hair analysis for detecting drugs of abuse, including improvements in sample preparation techniques. Kim et al. (2020) demonstrated the application of direct analysis in real-time-high-resolution mass spectrometry (DART-HRMS) for rapid screening and identification of drugs and their metabolites in hair samples. This approach allows for the simultaneous detection of multiple chemicals without the need for lengthy sample preparation [14-17].

Segmental analysis is the process of examining various sections of hair to identify the pattern of exposure over time, which can reveal crucial details regarding the frequency and severity of the exposure. Micro Segmental Hair Analysis (MSA), which removes individual hair strands at intervals of 0.4 mm, or roughly one day's worth of hair development, was created in 2016. This technique provides thorough information regarding the process of drug intake into hair as well as strong proof of drug use in investigations of drug-related crimes [18].

The isotopic composition of elements in a hair sample can be determined using Isotopic Ratio Mass Spectrometry (IRMS), which can provide information about the person's geographic origin and the substances to which they have been exposed. This method has enormous potential for use in forensic investigations as well as studies on environmental exposure.

Hair analysis has been used to uncover metabolic fingerprints connected to drug or chemical exposure through the study of tiny molecules (metabolites) produced by cells and tissues, or Metabolomics. This method can offer a more thorough and in-depth evaluation of exposure as well as insight into the toxicity mechanism. Hair possesses enormous potential as a metabolomic sample for monitoring chronic diseases. A study was conducted aiming to investigate the metabolic alterations in hair to elucidate a suitable treatment modality for Methamphetamine use disorder. Consequently, hair samples taken from current and previous methamphetamine use disorder patients underwent both targeted and untargeted metabolomics analysis using mass spectrometry. A similar approach can be practiced in conducting research on exposure to other drugs of abuse [19].

We can now detect and quantify drug and toxic substance exposure, as well as comprehend the long-term implications of this exposure on human health, thanks to advancements in the analytical toxicology of human hair. In the upcoming years, these techniques are probably going to keep developing and getting better, giving us new perspectives on the intricate connection between environmental exposure and human health [20].

A current field of study in the toxicology of human hair and nails is the identification of new pharmacological targets. Recent research has concentrated on finding new psychoactive compounds (NPS) in hair, nails, and other body tissues. Examples of NPS include synthetic cannabinoids and cathinones. The importance of detecting NPS in hair and nails has grown because of their pervasiveness in the drug market and their possible health dangers. Depending on the length of the hair & nail sample taken, drug and toxin testing is a viable approach for identifying drug usage over an extended period of time, which can range from weeks to months or even years [21,22].

The general timeline for finding drugs in human hair & nails is summarized in Table 1.

Table 1. Detection of Drugs in Hair and Nails [22,23,24]

S.No.	**DRUG/TOXIN**	**Metabolites**	**Minimum Detectable Levels**	**In Hair**	**In Nails**
1.	Cocaine	Benzoylecgonine and ecgonine methyl ester	0.5 ng/mg	Up to 90 days after use	up to 6 months after consumption
2.	Marijuana	11-Nor-9-carboxy-delta-9-tetrahydrocannabinol (C-THC)	0.1 ng/mg	Up to 90 days after use	up to 6 months after consumption
3.	Opioids	Monoacetylmorphine, Morphine, Codeine	0.2 ng/mg	Up to 90 days after use	up to 6 months after consumption
		2- ethylidene- 1,5-dimethyl-3,3-diphenylpyrrolidine, (EDDP)	0.5 ng/mg		
4.	Amphetamines	Methylenedioxyamphetamine	Hair- 8.0ng/mg Nails-9.7ng/mg	Up to 90 days after use	

	E.g.: Methamphetamine and Adderall	3,4 methylenedioxymethamphetamine	Hair-53.4ng/mg Nails-60.2ng/mg		up to 6 months after consumption
5.	Benzodiazepines (such as Xanax and Valium)/ Non benzodiazepine hypnotics/ Antidepressants etc.	Venlafaxine	Hair-44.31 ng/mg Nails-7.02 to 22.6 ng/mg	Up to 90 days after use	up to 6 months after consumption
		Zolpidem	Hair-0.16ng/mg Nails-0.40 to 1.42ng/mg		
6.	Alcohol or its metabolites	Ethyl glucuronide (EtG)	Hair-2.1 to 3.5 pg/mg Nails-2.3 to 23 pg/mg	Several months after consumption	up to 6 months after consumption
7.	Heavy metals such as Lead, Mercury Etc.	Depends on the type of heavy metal poisoning.	Variable based on techniques used	Up to 12 months after intake	up to 12 months after consumption

It is important to note that hair drug testing has a longer detection window than urine or blood testing for drug use. This is because when hair grows, drugs and their metabolites get trapped in the shaft, extending the detection window. Human nail drug testing can offer a wider detection window than hair drug testing. Since nails grow more slowly than hair does, and nails are four times thicker than the typical strand of hair and often capture more substances than hair can, drugs and their metabolites get more deeply impregnated and are therefore more detectable for longer periods. But one must consider that the detection window can change depending upon several factors including the user's rate of hair and nail growth, the dosage, exposure, and strength of the drug, and the accuracy of the test results which can also be impacted by hair treatments, nail polishing, use of cosmetics and also coloring of nails and hair [25].

Due to their toxic effects on hair follicles, poisonings brought on by substances like arsenic, thallium, and heavy metals can result in alopecia. For instance, arsenic interferes with the cellular processes necessary for hair growth and can cause premature hair loss. The production of keratin, a protein necessary for the growth of new hair, is inhibited by thallium poisoning, which causes hair loss. Additionally, cancer chemotherapy is also a cause of alopecia. In such cases, it is difficult to obtain hair samples for analysis. Several nail pathologies can impede the utility of nails for toxicology purposes. Onychomycosis, psoriasis, and chronic paronychia can induce structural abnormalities, thereby hindering the acquisition of dependable samples for toxicological analysis. Furthermore, significant nail trauma, such as nail bed lacerations or extensive nail matrix impairment, can compromise the nail's integrity, thereby impacting its suitability for toxicology testing.

To guarantee the accuracy and reproducibility of the results, validation, and standardization of hair and nail analysis methodologies are essential. The quality control and inter-laboratory comparability of the results have been enhanced with the establishment of standardized protocols and guidelines for hair and nail analysis, such as those from the Society of Hair Testing and the Society of Forensic Toxicologists.

The creation of novel analytical methods and the fusion of various OMICS approaches will determine the future direction of human hair and nail toxicology studies for medico-legal purposes. It is anticipated that improvements in metabolomics and imaging mass spectrometry will help find new biomarkers for drug exposure and give spatially resolved data on drug distribution in hair and nails. [26].

The application of Forensic tricho-toxicology and Forensic onycho-toxicology in Forensic Medicine is as follows:

1. Hair and nail samples are of utmost importance in cases of an autopsy upon exhumation to rule out heavy metal criminal poisoning. A routine practice of collecting these specimens for chemical analysis should be considered in all exhumation cases where it is felt necessary.
2. Substance abuse assessment in living and dead, especially in cases of drug-facilitated acute/chronic sexual assault/abuse. This shall become crucial evidence if the timing of exposure to drugs could be ascertained with certainty.
3. Occupational exposure assessment to various industrial toxins in both the living and the dead. This has a wide range of occupational safety and health applications and aids in the process of establishing a temporospatial association between the toxin and disease.
4. Environmental exposure assessment to various toxins can be easily identified by using nails and hair as substrates. This has an application in forensic medicine in investigating chronic accidental poisoning as a cause of death.
5. The toxicological analysis of hair and nails may provide drug abuse-related insights into investigating sudden deaths in young individuals, where no other cause is apparent at autopsy.
6. Autopsy surgeons may also consider the role of hair and nails as routine samples for chemical analysis to identify the cause of death in poisoning cases.
7. The forensic taphonomy study of hair and nails may also provide insights into antemortem exposure to various toxic substances.
8. The cause of death due to chronic damage caused by antipsychotics/antidepressants amongst psychiatric patients can be ascertained using the toxicology of hair and nails.
9. In all cases where a corpse is found in an advanced state of decomposition, it is advisable to preserve hair and nails for chemical analysis as a matter of routine because they constitute a relatively fresh sample for analysis even days after death.

The toxicology of hair and nails may also provide further insights into therapeutic drug monitoring and chronic disease management in the future.

It is also pertinent to mention that over-interpretation of the toxicology of hair and nail data can sometimes make us miss the forest for the trees. An autopsy surgeon should cautiously interpret these results in accordance with the objectives of a medico-legal autopsy.

Conclusion

In conclusion, the field of toxicological investigation utilizing human hair and nails as substrates is rapidly advancing. Recent developments in sampling and preparation techniques, as well as our understanding of drug metabolism and disposition, identifying new therapeutic targets, and establishing validation and standardization protocols have greatly improved the reliability and reproducibility of results.

Future research efforts are expected to focus on several areas to enhance the utility of hair and nail analysis in forensic toxicology. Firstly, the development of innovative analytical methods will be crucial in expanding the range of detectable substances and improving sensitivity and specificity. Additionally, incorporating OMICS methodologies, such as genomics, proteomics, and metabolomics, into hair and nail analysis holds promise for gaining a deeper understanding of drug exposure, metabolism, and individual variations.

These advancements will contribute to the continued growth and application of hair and nail analysis in forensic toxicology, enabling forensic scientists and practitioners to obtain valuable information on drug use, exposure to toxins, and other relevant factors. As research progresses, the field will continue to evolve, providing even more accurate and comprehensive insights into forensic investigations and contributing to the advancement of forensic medicine.

Conflicts of interest

The authors declares that they do not have conflict of interest.

Funding

No funding was received for conducting this study

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