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Elemental Composition of Hair and its Role in Forensic Identification

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Summary

A blend of microscopic assessment of hairs using various identification techniques and further extending to DNA examination will provide the best achievable detailed information about the questioned hair samples. Because of the availability of various new technologies and their involvement in forensic science and legal investigation system including nondestructive as well as destructive nature of identification methods like EDXRF, EDS, ETAAS,GCMS, HPLC, SEM, SEMEDS, NAA, microscopy, both mt and nuclear DNA techniques and also protein profiling. Due to destructive nature of various techniques like DNA analysis, EDS, SEM ICPMS etc microscopic and other non-destructive analysis must be conducted, followed by the DNA examination, because the segment of the hair used in DNA analysis is a destructive technique thus it make hairs unavailable for the microscopic examination. Modern hair examination techniques provide information regarding hair origin, sex, age, habit, species, environmental exposure, geographical parameters, elemental composition, metal toxicity and also congenital abnormalities associate with genetic abnormalities and results of hair analysis will provide vital information regarding its involvement as physical and biological evidence as well.

Introduction

Hair is among most common types of evidence reported in criminal investigations. Throughout the stages of usual hair-growth parameters, hairs goes through a continuous loss from every individual, and due to this loss these hairs may be transferred from one individual to another in any criminal commotion [1]. Hair have gained a wide interest in its examination for various fields counting taxonomy, zoology, wildlife, clinical biology, dermatology and forensic investigation [2] due to its non-invasive and quick analysis along with précised and accurate results. Hair examination was in routine practice since last decades for species identification and dermatological uses but from last decade hair examination for clinical detection of various diseases [3] and for race, sex, age, and occupational identification has gained center of interest for forensic scientists/examiners [4]. Hair can be used in identification of environment exposure, food habits, soil and geographical parameters, in detection of criminal offences related to wildlife poaching of protected animals [5] and also in detection of metal poisoning [6]. In recent years mitochondrial and genomic DNA from hair has opened wide vistas for forensic investigations [7] which lead to the investigation of not only cases related to disputed paternity [8] and species identification but due to mt-DNAs hair examination has received interest of scientists in the field of evolutionary studies due to its matrilineal nature [9]. Has provides very useful information in cases of rape, sexual assaults, murder, illegal wildlife trade and so on [10]. The forensic evaluation of hair can be very important in the physical evidence examination and assessment by representing the association between the suspect flanked due to a crime scene or a victim and a suspect or representing that no evidence exists for an involvement connecting a criminal with a crime scene or a culprit with a victim [11]. Even though the science of microscopic hair investigation can never result in identification [12], the final aim of any forensic examination is to give statements on the bases of examination and scientific observations that will provide information about criminal and crime scene connection with crime in court of law or to any other agency incorporated in the investigation [13,14]. The motto of this document is to analyze the bases of microscopic hair analysis and comparison including the analysis and comparison of the morphological uniqueness present in hair. Based on this morphological uniqueness suspected hair is examined for its origin identification weather from human origin or belongs from any other species [15,16]. Within each of these two groups, further information concerning the potential donor can be obtained using this same microscopic individuality. Finally, an assessment can be conducted for an unknown hair with a known reference hair sample [17].

Hair Composition

General structure

Hair and other fibers of animal origin mainly composed of three different layered regions- cuticle, cortex and medulla. Outer layer cuticle shows scales arranged like tiles which is differentiated in two parts, (a) inner endocuticle with a pitted honeycombed structure with ridges which opposes digestion by trypsin, and (b) outer exocuticle which is smooth, featureless and tryptic digestable, along with these two layers a chemically inert epicuticle is also located around the scales [18]. The main constituent of the hair is cortex composed of cigar-shaped cells which varies in size according to keratin type [19]. Cortical cells are formed by fibrils and are associated in sheets which are further embedded in amorphous material where fibrils are composed sub-fibrils responsible for X-ray pattern given by protein [18,20]. However , all fibrils are not identical but it has been demonstrated that a fiber may be differentiated in two hemi cylinders longitudinally termed as paracortex and orthocortex from which orthocortex is more reactive and attains dyeing readily than paracortex [19,20]. Sub-cuticle, an intermediate continuous membrane surrounding the cortex is present which is more resistant to chemical attack than remaining fibers. Keratin fibers have honeycombed structured protein containing air pockets running through cortex either continuous or intermittently called medulla where sulfur is deficient in this part of keratin fiber [17,21-23].

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Chemical composition

The hair and related keratin are fundamentally composed of the condensation of α-amino acids; these are linked in a plane by electrovalent salt linkages, and by covalent cystine linkages, between hydrogen bonding within suitably arranged peptide groups and with polar side charged chains and in other by less Vander Waal forces [24]. Fibers are not, however, uniform and two regions- amorphous and crystalline have been differentiated. The segment of the hair responsible X-ray photographs of keratin is some 20% crystalline in nature [25-28]. When this keratin fiber is stretched, it gains the structure of fully extended peptide chains as observed in stretched silk therefore it was concluded that the peptide chains of unstretched α-Keratin are folded and when the fiber is stretched to give the β-form of keratin where the folded chains are made straight [29-32].

The pigmentation of animal fibre

Melanin is mainly responsible for the many shades of black, brown and other shades of hair and is found in the form of granules which are seldom uniformly distributed in fibre or in crimpy fibres the pigmentation is asymmetrical [33]. More pigmentation is observed in paracortex than orthocortex, this asymmetrical distribution of granules is originated by differential allocation of melanocytes inside the follicular bulb where the fibre is formed [34]. Electron microscopy of the melanin granules revealed their egg-shaped bodies; the ratio varies in major and minor axis with the origin of granules. The Pigments get deposited over the layers of the granules in hair made up of layers formed of proteins [35,36]. Electron micrographs showed the axis of granules tends to lie parallel to the axis of cortical cells, and granules are arranged end to end on rows covered by fiblillar keratin. Melanins are chemically inert in nature and are polymers of high molecular weight and known to be formed due to the action of tyrosinase to yield indole-5-60quinone which then polymerizes [37]. Several researches have revealed that natural melanins show close association with metals, especially iron and with proteins [38-40].

Effect of Age, Sex and Color on Composition

A research on south Poland revealed that the hairs of children below age 15 showed a tendency to have more levels of sodium, potassium, phosphorus, aluminum, boron, lead, chromium, gold, platinum, beryllium and tungsten and less calcium, cooper, vanadium, tin, barium, silver and zirconium than hairs of older people [41]. Hair of individuals from age of 15-25 contains more calcium, zinc, silicon, magnesium and strontium and lower amounts of sodium and potassium whereas adults in the age of 25-45 showed higher percentage of calcium, silver, and zirconium with lower levels of sodium and potassium. Analysis of people of age group 45-65 years revealed lower amounts of calcium, zinc, magnesium, strontium, silver and zirconium and higher amounts of sodium, potassium, cooper, vanadium and tin [42-44].

Research studies on hair color by chojnacka and his co-researchers revealed the differences between the hair samples having various colors and suggested that naturally colored hairs have less amounts of Sr, Ba, Ca, Mg, W, Mo, Ag and Mn than in artificially colored hairs and generally, artificially colored hairs contains more of all elements. In naturally colored hairs the highest amount was of Si, Ni, Cd, Au, Sb, and lowest are of V and Mo in dark and blond hair. When comparing the effect of sex it was observed that male hairs contains more amount of lead and females have highest amount of Ca, where Co content has no significant variations and following results were observed in common as Ni content is higher in females and males have higher amounts of Cu and Pb. Higher amount of lead is observed in children whereas older ones have lower amount of Zn and cadmium was not having any functionality depending on age, sex and colour [45-47].

Absorption of the Elements in Hair

The adsorption of elements on hair like zinc, lead depends on the acidity of the hair as hairs are ion exchanges. Alkaline metals were loosely bound whereas alkaline earth metals are bounded at a great extent observed during removal of metals from hair in detergents. Researches revealed that hair absorbs metals greatly in alkaline pH whereas decreasing the pH below 4 starts elution of metals to some extent. Acidity of the hair depends on negative and positively ionizable groups present on the hair which is influenced by hair treatments, where waving effects greatest and the samples with permanent waving showed higher adsorption rate for metals. Calcium levels were increased on the part of hair shaft after waving that may be due to tap water calcium adsorption on the hair. The findings on hair research suggested that hair is an ion exchanger, with pKa between 4.5 to 5.0 which is relatively close to 4.25 of "y-carboxyl or 3.7 of 13-carboxyl and far from sulfhydryl and sulfonyl group i.e. 10.28 and 1.5 respectively [48-49].

Hair-Metal Binding

Metal analysis concerning process of incorporation of metals including biochemical and surface binding had provided significant clues in identification of metal toxicity and deficiency of essential metals. Many essential elements are required for the synthesis of proper structure of keratinized hair fiber where some are incorporated as surface binders and remaining get involved from biological transport from the body hair surface is considered as a binder for metals and the shaft is proteineous in nature which contains about 11-18 percent of sulfur-containing amino acids that may be cysteine and its dimmers cystine and trace amount of methionine sulfur is also present [50]. The shafts over the skin layer is metabolically inactive and have a layer of cuticle cells above cortical cells which results in formation of bulk hair shaft and a central core of the medullar part. The cells in the hair degenerate and show a continuous loss of the elements present inside the skin layer [51]. The persistansive characteristic to metal and sulfur bonding is supported by extractability of the metals from hair to be nonconsistent with sulfur binding. In general sulfur-metal bonds are not stable as evidenced by the reaction between dilute acid and Zinc Sulfide due to formation of hydrogen sulfide and Zinc is used in formation of monosulfides used in laboratory production of cysteine and ferric catalyzed oxidation of cysteine to cystine in basic pH whereas Zinc and sulfur binding had proved by libration of Zinc in acid in case of soluble proteins, where Mercury-Sulfur bond is highly stable. Sulfide of cooper is similar to mercury-sulfur bond except a slight degree of separation where mercaptan provides stability to cupric sulfide. Silver-Sulfur bond is also slightly unrelated to the cooper-sulfur and mercury-sulfur bonds which only lean removal of added silver from hair was reported by bate. Other tests had specified the association of cooper with hair including non-sulfur bound cooper in hair [52-53].

Hair shows a significant higher absorption of elements at pH-6 then pH while, before this experimental repot carboxyl groups was assumed to be responsible for metal binding as unbound carboxyl groups were more protonated in lower pH media rather than towards acidic pH and shows a small amount of carboxyl amino's binding with cations of metals but Sulfur-Metal bonds could affect not only carboxyl involvement, in protein polymers alpha-carboxyl groups of amino

acids are bound in amide formations and hair contains for about 19% if dicarboxylic amino glutamic and aspartic acids. Serine hydroxyl group and some nitrogen group's dipole bonding are also possible and some anionically charged non-metals were separated in hair to find more metals at lower pH [54].

When mare carboxyl anions on hair would be neutralized by protonation. The incorporation of metals to hair occurs in hair bulb due to secretary mechanism inside skin or at surface of skin. The cuticle layer of hair contains sulfhydral groups longer than inside cortex.

Metal incorporation may also occur directly at keratogenous zone above the hair bulb which results in the presence of cystine in sweat which includes the secretion of sweat and sebaceous glands. Sweat has noncrediated as a hair metal content factor where as a significant loss of some trace metals have been reported. Sebaceous glands are attached to hair canals just inside the skin layer where the sebum is secreted by gland which is incorporated by keratinized hair shaft. Reports revealed more accumulation of elements in growing hair than in stagnant hair growths [55-57].

Uses of Hair Analysis

Hair of all the organisms varies in a numerous ways including morphological appearance, physiology, genetic makeup, elemental composition and trace elements. Animals from same family have similar characters then with others. Taxonomists and forensic examiners search for identification of these differences for using the results in differentiation and to classify the organisms for solving crime and taxonomic issues. Not only for forensic examiners hair have played a vital role for wildlife biologist, archaeologists, anthropologist and textile conservators. Many researchers have revealed the morphological hair characteristics using various microscopic techniques based on their medullary index and scale pattern using Scanning Electron Microscopy (SEM) as well as light microscopy. Scientists and forensic examiners have incorporated SEM and TEM studies in identification of hair samples from different species in cases related to wildlife crime, textile industry frauds, taxonomic classification and also in cases involving hair as a physical evidence either in identification of origin, or sex in rape assaults or in cases where domestic animals were involved. Hair can be used in identification of various crimes in ways to identify geographical location, elemental composition, species, sex, occupation, food habits and also the environmental conditions.

Microscopic examination

There are different characteristics associated with hair identification using three different anatomical regions present in hair: inner Medulla, outer cuticle and intermediate cortex. Cuticle is differentiated on the bases of scale pattern and also on the bases of scale layer difference using SEM and other parameters like width of the cuticle, the changes in the thickness throughout the hair, presence of the pigments. Nature of the cuticle margin may vary from smooth, looped, ragged to damage. The cortex can provide information regarding the density, organization, size, and distribution of the pigments and it varies tremendously between racial groups. The medulla also provide information regarding its pattern varying from continuous, discontinuous, fragmented to absent in hair. The presence of root cells provides the information regarding the force used to pullout the hair and the root point of the hair also provides information regarding instrument/ weapon used to cut the hairs [58,59].

Chromatographic examination of the hair

Chromatography of the hair provides the information regarding the use of hair oil, hair colors, natural herbs, dyes and also hair shower gels. The information regarding the hair oil analysis leads the identification of the suspects from their hair oil as well as sprays, gels and hair colors/dyes. Thin layer chromatography, high pressure liquid chromatography and gas chromatography are commonly used in this type of identification. Sometimes artificial treatment results in a line of demarcation that provides a notable change in colour along the length of the hair. Bleaching will results in removal of pigments [60].

Inductive coupled plasma-mass spectroscopy (ICPMS) examination

Interest in hair as a clinical sample as well as in forensic identification has increased in current years due to its advantages offered by human hair over other specimens like blood and urine. Hairs provides information regarding variations in trace elements by ICPMS will provide many advantages over other biological samples as the concentrations of almost all trace metals are found to be higher in hair then in other materials; specimens can be collected more quickly, easily and does not require special storage conditions like blood, tissue and other body fluids. Urine and blood provides short time responses while the elemental concentrations in hair provide a retrospective index of trace elements. Nitrogen and hydrogen peroxide digestion is followed in ICPMS which offers a significant method for confirmation of different metals present in hair those can be used to study the environmental exposure, smoking habit, feeding habits and also regarding the information of geographical area. ICPMS has been showed to be a superior method for the multi-elemental determination of minor and trace elements in hair tissue samples [61].

Energy dispersive spectrum and energy dispersive X-Ray fluorescence analysis

EDXRF and EDS analysis provides both weight and atomic percentage of the elements present in hair which lead to identification of species and helps in identification of the geographical area of the suspect or of the victim. EDXRF provides a non-destructive technique and requires a less amount of sample which can further be used for other identification methods. Although EDS is a destructive technique for identification but it requires very less amount of the sample and can be done from just 1-2 mm of the hair sample [62,63].

Electro Thermal Atomic Absorption Spectroscopy (ETAAS) analysis

Only 0.02-0.04 mg of the hair samples are required for the analysis and can be inserted directly in to the system without treatment in the platform of solid sampling auto-samplers. Provides a rapid identification with references and facilitates the quicker examination of the hair samples for identification of metals present inside hairs so that detailed information can be obtained regarding the occupational or environmental exposure and also provides information regarding the deficiency of trace elements which can further be of use in medical diagnosis [1,9,28,63].

Hair Analysis using Neutron Activation Analysis (NAA)

With growing industrialization and technological advancements, human exposure of toxic trace elements has become a major environmental issue as well as it has gained wide interest of forensic

investigator in identification for the cases related to acute and chronic metal toxicity. It is worldwide recognized that hair tissue serves as an accumulator for trace elements analysis and also play a vital role in criminal investigation by both types as clinical evidence in metal poisoning cases as well as in cases of occupational exposures. NAA provides a wide range of elements and is among quickest methods for hair elemental profiling [7,64,65].

Use of Hair in The Diagnosis of Heavy Metal Poisoning

Heavy metals are most frequently concerned in human poisoning includes lead mercury cadmium and arsenic and were in use since long time. Distant from their clinical symptoms, the effect on tissue and cellular level due to heavy metal poisoning, for example proteinuria due to mercury and basophilic stippling of erythrocytes in lead are useful in providing information as adjunctive evidence but because of uneven circulation of metals inside the body, the levels of these will varies in the blood and urine that is not sufficient to imitate the specific levels within the organ system. Notwithstanding this restriction, hair analysis is invasive and provides a large freedom in both humans as well as in animals and makes it an attractive alternative to both humans and animals for analysis of toxic metals [62-64,66].

Use of Hair as Bio Indicator

Hair is useful bio-indicator to estimate exposure of the environmental trace elements because of its easy collection and trace element reflection from long exposure durations. Although trace elements are not only incorporated inside it due to endogenous uptake but also from exogenous materials such as soil, dust, water etc and can be used as a bio indicator to study the exposure of various trace as well as toxic elements. Hair is composed of keratin and main elemental component is sulfur which found to be uniformly distributed all over the hair but the metals present on the outer surface/ surface metals provide a significant demarcation regarding the environmental exposure of that element over hair and does not penetrate into the internal hair [13,27,67].

DNA Analysis of Hairs

The tools in molecular biology have enabled investigators to identify biological evidences at the DNA levels, both mitochondrial and nuclear DNA. Materials including hair, which contain nucleated cells can potentially be subjugated using nuclear DNA fingerprinting. For nuclear DNA typing hair must have sheath material to be flourishing. Unlike nuclear DNA mitochondrial DNA in maternally inherited and is not unique to individual. Therefore, mitochondrial DNA has gained its wide acceptability in identification of species and its applications in cases of wildlife crime and taxonomic identification due to its COI DNA barcode region [11]. Kolowski et al. (2004) conducted a research for the evaluation of the accurateness of short tandem repeat (STR) Deoxyribonucleic Acid examination and microscopic assessment. Pubic-hair samples of at least 50 hairs were experimented from 27 volunteers working within laboratory. Laboratory volunteers were used because of presence of their DNA within the laboratory database. Using these samples, a nonaligned five different sets of four hairs in each sample made by a third party for using them as questioned samples. in each questioned hair sample, two hairs were of anagen stage, or vigorously growing, stage. Control hair samples, five hairs have taken and mounted on glass slides for microscopy. From the root of hairs those were of anagen stage have been removed from remaining length and proceeded for the isolation of the nuclear DNA, and that extract was assessed using STR typing technology. Short tandem repeat (STR) analysis of the unknown hairs have been done and identified correctly for the source of all the three of the questioned hair samples [4,9-11].

Role of Hair in Identification of Geographical Area

Many studies have been done by the researchers and revealed about the level of trace elements in hair reflected, the degree of ecological concentration like soil, water, food and metabolism. Higher concentrations of the elements in the environmental levels like soil, food intake, water and metabolism leads the individual for strong innate selection for tolerance of the elements. As ecological contaminants are of vital importance in forensic identification of cases where hair plays as key evidence in identification since last century.

The concentration of these elements varies between each geographical region which leads to different levels for accumulation of these elements in hairs. Animal hairs show significant variation in the concentration of these elements then humans due to their extended exposure to soil contaminants through feeds. In the current studies on forensic science, clinical and toxicological studies involve elemental analysis of hair with special reference to the identification of geographical region from hair elemental analysis. Felidae and have revealed clear significance of elemental concentrations in Felidae family of Gujarat state of India

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