Role of hair as Evidence in Investigation: A Forensic Approach

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Abstract— Hair and other animal fibres can provide vital clues and information after species identification, about the chemical composition, colour treatment, geographical region, growth rate, age, colour and feeding habits including different cosmetic effects. Examination of hair also provides the medical history of the individual regarding therapies, serological identification along with the conventional information including toxicological assessments and drug addiction testing. Morphological and other surface studies including transmission studies have proved the significance of hair in identification of the individual since last 5 decades in the field of the investigation based on their microscopic, elemental analysis and genomic DNA from hair root cells, followed by mitochondrial DNA examination from shafts where hair composition and its association with other environmental factors are major concern including alpha and beta keratin structures in the cuticle along with different melanin granule concentration in cortex, and medullary index. Hair examination has provided aids in identification of criminals in forensic and other legal investigations thus an attempt has been made in this article to provide an account on the various aspects of hair that can be used in investigation..

Index Terms— Hair analysis, Forensic investigation, Geographical region marker, Hair composition

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INTRODUCTION

Hair being the most common biological material found at the scene of crime, plays a crucial role in criminal investigations related to wildlife, taxonomy, Investigative dermatology, pathology and other applied fields of forensic science[1]. As the growth is continuous from birth of an individual till death, there is constant loss and replacement of the hair strands spread over the body so they get transferred during the act of a crime [2]. Its microscopic comparison and chemical analysis plays a very significant role other than blood and fingerprints. Hair is usually used to study characterisation of the known sample versus the questioned hairs recovered from the crime scene to check if they are from a common source for the establishment of a relation between crime and the criminal. Hair identification is done non invasively followed by invasive examination and it provides very precise results along with other relevant information about the suspect and proves its utility over other evidences in the detection of drugs, other illegal substances along with the information of habits and geographical region. Presently it is a widely accepted tool to identify the age, sex, colour, race, disease profile, diet, occupational and environmental exposure, metal poisoning, geographical indicator, illegal wildlife trade, sexual assault, rape, disputed maternity and paternity matters[3,4] and in cases of mitochondrial DNA examination where the questions are raised related to evolution and inheritance[5]. Hair also provides the information related to the poisonous or toxic substance that may have been linked to health or problems in psychological, reproductive disorders and in developmental toxins. Hair is always considered to be associative evidence and its ultimate objective being to associate a suspect and the victim in the act of crime supported by evidences based on the scientific approach and the crime scene scenario [6,7]. The present article represents an account on recent advancements and techniques in forensic hair characterization for its morphology and distinct identification parameters as being unique to every individual for the forensic examination of hair.

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COMPOSITION

Hair is a fibre, thin structure approximately around 0.1mm in diameter with an oval or a circular cross-section.[8] It is mainly composed of three concentric regions i.e. the outer layer being the cuticle which is a thin coating covered by tilted scales; the main constituent being cortex contributing to almost 90% of the total weight of the hair made up of differently shaped cells specific to the hair type [9] and, the keratin fibres are arranged in a honeycombed arrangement of cell structure containing air pockets continuously or discontinuously, called the medulla.[10,11] The follicle is the most active part which lies beneath the skin surface producing keratin proteins and the hair shaft collectively make up the hair strand. The follicle associated with the hair shaft acts as a mould to shape the strand. The hair shaft is stiff to a greater extent that helps reduce damage caused due to shear and stress in the strand. The frictional behaviour is mainly linked to the cuticle which along with the cortex also determines the tensile strength and smoothness of the hair[12,13] Condensation of alpha amino acids in the hair, are bound together in same plane due to the forces contributed by electrovalent salt linkages, covalent cystine linkages hydrogen bonding and Vander-Waal force of attraction[14]. Hair fibre is not uniform in composition and it is made up of both crystalline and amorphous regions. The segment of hair that captures the X-ray photograph of the keratin content is found to be around 20% and 80 % crystalline and amorphous respectively in nature.[15-18] The keratin fibre is chemically composed of two forms i.e., α-keratin and β-keratin. The α form tends to remain folded but when it is stretched, it corresponds to the β-keratin form [19-22].

AGE, SEX, AND COLOUR DETERMINATION USING HAIR

The melanin granules and its variants are responsible for the greying or any other discolouration to hair. The melanocytes are formed in the bulb of the follicle. The growth of hair is a cyclic process which occurs in 3 stages ie, anagen, catagen and telogen. The papilla cells are firmly embedded in the dermal layer of the scalp thereby transferring the nourishment to the hair strand. During catagen, the supply of nourishment decreases causing the strand to shrink thereby the follicle pushes them outward which follows the telogen stage where the hair is forced out by the newly growing strand from the subsequent anagen stage. Decreased melanocytes formation in catagen stage, results in greying of hair [23].In the region of keratinisation, the melanin pigments are instilled into large granules. Grey hairs are much wavier and coarser than heavily pigmented hair and white hairs contain more medulla.[24] Less pigmented hair like blond, grey or bleached hairs are more sensitive to light radiations and posses lower tensile strength than heavily pigmented hairs. The lipids i.e. the sebaceous gland secretions and the hair matrix are mainly responsible for dryness or the oiliness of the hair which are present almost all over the body.[25]

Artificial colouring agents have a direct influence on the components of hair due to increased exposure of chemical treatment during colourization. Natural hair have less amounts of calcium, barium, Strontium, magnesium, silver , molybdenum and tungsten as compared to artificial coloured hair which more or less contains all of the above components. Natural hair contains high amounts of nickel, silicon, cadmium, gold and tin and least amount of vanadium and molybdenum as seen in dark and blond hair [26-28].

The age of the person can be roughly estimated by observing the hormonal influence and it was found to give distinct results for different age groups. The diameter of the hair increases rapidly during the early years till teenage. Hair thickening, hair greying, coarseness and thinning of hair are the most common changes that can be observed. The diameter of the hair fibre decreases with increases in age that can be identified in many cases.

The hair follicle and skin cells are having sebaceous glands and posses glandular secretions, the constituents of which are directly related to the age and sex of an individual. Before the onset of puberty, the output from these glands tends to be low and increases through the teenage years till almost two decades. Beyond the fourth decade, sebum secretion decreases more in females than in males. Changes in the amount and composition of hair lipids results in lesser scalp hair density, lower growth rates and fibre diameter, cross section, greying and curvature with increasing age.[25] Studies have revealed that children below the age of 15 years had more of potassium, phosphorous, sodium, beryllium and tungsten and decreased amounts of calcium, copper, vanadium, tin, barium, silver and hairs as compared to older people[29]. Higher amounts of calcium, zinc, magnesium, strontium and lower amounts of sodium and potassium were found in the hairs of individuals in the age group of 15-25 years. Higher percentage of calcium, silver, zirconium and lower levels of sodium and potassium were seen in adults between 25 -45 years . People above 45 years to 65years of age observed to have lower amounts of zinc, calcium, strontium, magnesium and zirconium and higher amounts of sodium, potassium, copper, tin and vanadium[30-32]. By estimating the quantities of the metals that the hair contains, a rough estimation of the age can be derived. When it comes to sex differentiation, male hair contained more of lead and copper than females who had high amounts of calcium and nickel[26]. Cobalt did not show any significant variation while children were said to have high amount of lead, the older people had lower amounts of cadmium and zinc and it did not show any pronounced effect on factors of sex, age and colour [27, 28].

OCCUPATIONAL EXPOSURE

Hair fibres posses' minerals and other elements throughout the shaft. These elements are incorporated in hair by two mechanisms i.e. by biological metabolism and surface absorption respectively due to the sulphur and other metal associations [33]. The mineral content of hair fibre by natural biometabolism is estimated to be very low (less than 1%),making it difficult to identify if the inorganic matter is from an external source or it is arisen naturally[34]. The trace materials that were reported in hair are Calcium, Magnesium, Strontium, Sodium, Aluminium, Boron, potassium, Copper, Zinc, Iron, Manganese, Gold, Silver, Arsenic, Mercury, Lead, Tin, Titanium, Tungsten, Vanadium, Molybdenum, Iodine, Phosphorous and Selenium which are primarily found to originate from sweat deposits.[35] The actual origin of these elements may be due to biological or environmental factors and occupational exposure on people are more related to their environment. Water supply provides large amounts of Calcium, Magnesium, Iron, Manganese and copper to the hair. Other influences include, diet, air pollution and metabolic imbalances [36]. Transition metals on hair can be internal and external. Copper and few other elements are reliable indicators of consumption through diet [37] but Iron and calcium are obtained by external influences. Therefore, iron and calcium are more readily said to be thrown off balance than Copper. The two very important metabolic processes are keratinisation of human hair that oxidises thiol to disulphide and in the oxidation of tyrosine to melanin in the presence of tyrosinase and both make use of copper in their reaction [38]. Other external source of copper can also be through water, producing green hair phenomenon in which the hair turns to green colour due to high amounts of copper in the water source. The endocuticle, the cell membrane complex and the medulla have regions of high carboxylic acid content and are likely to form divalent or trivalent metal bond more readily. The pigments of hair contains high metal content of which red hair contained highest amounts of iron (up to four times more) as compared to other colour hairs while the copper and zinc bound to both black and red hair melanosomes [39]. The pigments of human hair are also capable of producing hydroxyl-free radicals those can also be used for the comparison of occupational exposure on the basis of availability for corresponding element in working environment.

EFFECT OF HABITS ON HAIR

Other than the surroundings, imbalance in the diet like mal-

nutrition, smoking, consumption of alcohol has direct influence on the hair [40]. The protein composition is influenced by the diet and affects the ratio of sulphur proteins that determine the hair fibre curvature. Alternatively, diet supplementation can influence the protein composition in the human hair. Cystine, arginine, methionine largely control the protein balance in human hair [41] Drugs and their metabolites are studied in detail as they produce some by products that are transferred to the scalp hair strands. This can be used in investigations for the detection of drugs doping and other relevant substances of forensic importance. Also, exposure to broad spectrum antibiotics and pollutants can be identified by means of hair analysis [42]. Trace metal analysis on hairs have many advantages as hair elements of toxic nature correlated with many disorders and habits like smoking and consumption of alcohol[43]. Hair of smokers contained more Zr, Mo and As while that of non smokers contained more of V, Se, P, Sr, Si, Ba, Na and Cr while the level of Cd, K, Ag, Ca, Co and Be was very similar in proportion. Smokers have high amounts of Cd in their blood and urine [44]. The highest amount of Cr in smokers has been derived from the soil which is later on picked up by the tobacco leaves.[45] It also reflects long term history of individual exposure. It involves non – invasive and non-destructive procedures and can deliver valuable information about the person's health, drugs and diagnosis of certain diseases [46].

AS INDICATOR OF GEOGRAPHICAL REGION

Concentrations of metals such as arsenic, mercury, cadmium and lead obtained by the influence of the environment are almost evenly distributed with same amounts in internal organs. Hence, hair also acts as a very important diagnostic tool. It can also be observed that highest amounts of these toxic metals in humans are normally found in endoskeletons like hairs, nails and skin. Heavy metal contamination maybe due to industrial activity and hair may identify and diagnose the toxic element. Air pollution is also a very influential factor. More complicated disorders can also be studied by analyzing the human hair. It finds application in various purposes to estimate the level of nutrition and biological monitoring of occupational and environmental exposure to heavy metals. More accumulation of elements can be seen in growing hair than in stagnant hair growths. Studies conducted by Dahiya and Yadav [47-48] revealed the effect of elemental composition for production of forensic geographical markers (FGM) and have been established from the hairs of *Panthera leo persica*, *Panthera pradus fusca* and *Panthera tigris tigris* from different geographical areas and processed under Scanning Electric Microscope coupled with Energy Dispersive Spectrograph and Energy Dispersive X-Ray Fluorescence followed by Differential Scanning Calorimetric Analysis which were used for elemental and thermal analysis respectively. Hence, FGM helps in narrowing down the possibility of the animal being present in that area by analysing the amounts of metal in soil and water. [49,50]

DISEASE HISTORY

The health condition of a person can be observed by elemental analysis followed by studying the speed of its growth and fallout. Hair loss may be due to medication, allergies, hormonal imbalances, improper hair care, diet and family history which may accelerate its growth to the telogen stage leading to unusual hair fall. In cancer treatment, 'Anagen effluvium' is the condition of hair loss following chemotherapy because of metabolic imbalance leading to abrupt decrease or no reproduction of matrix cells resulting in the loss of dystrophic anagen hairs.[51]

ROLE OF HAIR FOR CRIMINAL PROFILING

Almost every cell type in the human body is nucleated and made up of chromosomes which are majorly made up of nuclear DNA while the mitochondria of the cell is having mitochondrial DNA which is purely matrilineal in origin. Earlier, studies were conducted primarily based on microscopic examinations while later on the focus was shifted to derivatives of proteins using ATRFTIR because of its non-invasive nature [52]. For DNA, It had a set back in terms of the nature of the sample obtained as its analysis required the root segment of the hair or tissue which may not be always present when the evidence is collected.[53] But then mitochondrial DNA has opened the new horizons for DNA identification from the shafts. The nuclear DNA does not always adhere to the strand due to the process of keratinisation, but in case of mitochondrial DNA, it can be found in hair shafts and are very resistant to damage [54]. In the actively growing phase called the anagen, the hair roots contain large amounts of nucleated cells in the root and surrounding sheath material. Most commonly encountered hair samples are from telogen follicles which without the follicular tissue may not be suitable for DNA analysis as they lack nucleated cells but, may contain sufficient amounts of mt-DNA in their roots and hair shafts for analysis. Microscopic techniques and DNA analysis are often complementary because the latter being a destructive technique consumes portions of hair and cannot be reused again. Microscopic analysis should hence be done prior to its degradation. In some cases, microscopic hair examination and comparison may be inconclusive as hair is fragmentary while the known sample may have been collected many years later than the questioned hair samples [55]. DNA analysis is not necessary when microscopic examination concludes as to coming from the same source. People of the same maternal descent will have similar mitochondrial DNA therefore, further analysis of the evidence is necessary to narrow down the individual from a family group. In products derived from wildlife artefacts like leather, skin etc, which are being treated up to very high temperatures for their processing these results in DNA damage while the mt.DNA remains stable until a very high temperature variation is achieved due to the thermo stability and heat tolerance capacity of keratin fibres. So by further hair shaft examination for mt-DNA fingerprinting the evolutionary history and species identification can be done using Cytochrome b Sub Unit-1(*COI*) [56].

SEROLOGICAL STUDIES

Hair carries the glandular secretions deposited over it thus it is in contact with body fluids like sweat, sebum and other hormones. These glandular secretions also contain biological

elements like blood grouping substances and other cellular metabolites which can be used to isolate the information regarding serological examinations and for the antigen and antibody assays like ELISA & RIA. These secretions located over the hair may also contain the external evidences related to poisons and other plant extracts which can be used in the identification of the species of origin.[46] In some cases, these scalp hair contains the saliva of other species of animals that may have come from their pets so we can identify them on the basis of serological examination of hair.[49-50]

HAIR AS A BIO-INDICATOR

Hair is an excellent tool to assess changes in the body of an organism indicative of the influence and cause [57]. Determination of occupational or environmental exposure, drugs and other toxicants, medical changes on a person directly reflects and manifests by characteristic changes in hair, hence acts as a highly reliable bio indicator in forensic applications [58-60]. Trace metals and medical history can be studied and is a non-invasive method of examination.[61] The amount of metals may also vary due to seasonal and bodily changes[62]. Nails are also parallely analyzed as they are less prone to external contamination and both exhibit similar results. Techniques like atomic absorption spectrometry, ICP-OES, ICP-MS, SEM, X-ray fluorescence, proton induced x-ray emission [63] and LAICPMS are widely used for elemental analysis of Pb, Cd, Ni, Fe, Zn, Cu, Cr and U on a single hair strand. [64]

POST-MORTEM INTERVAL (PMI) ANALYSIS ON HUMAN HAIR

The most challenging identification a forensic Scientist/pathologist faces is to establish the time of death which they often determine by identifying the stage of decomposition [65]. Recent studies have been known to accurately determine the PMI using the scalp hair [66]. Longer it takes to examine the body since death; it is more likely that it gets influenced by environmental factors along with anatomical changes. It also plays a crucial role to establish the timing of assault and death to narrow down the alibi and the motive [67]. When there is a killing, it is very essential to study the chain of events and to identify if the person was alive or dead before being inflicted. Evidences are usually collected from the body, environmental and associative evidence, and based on the habits and everyday activities of the individual. Many physico-chemical changes take over shortly after death in a sequential order with its own time factor until the body gets disintegrated [68] Hair from the same individual show uniformity with respect to cuticle damage, proximal end morphology and fungal growth characteristics as a whole head hair from the same individual show uniform detoriation [69]. The fungal growth and changes in proximal end morphology are directly related to the PMI while cuticle damage is non significant. The slow decomposition rate of hair as compared to other softer tissues makes it a significant information source in older cases for investigation [70].

CONCLUSION

Hair examination can be used for the identification of various aspects of elemental composition, protein structure, geographical region, feeding habits, growth stages, medical history and various cosmetic treatments along with genomic and mitochondrial DNA and other serological examinations which can be used for the identification of individuals in forensic investigations and other conservation related strategies for society and criminal justice system.

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