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Utility of Human Scalp Hair as Biomarker

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Abstract: Human biomonitoring (HBM) is a scientific technique that allows us to assess whether and to what extent environmental pollutants enter humans (Alves 2014). The technique relied on the various biomarkers like blood, urine, nails and hair. The blood is invasive biomarker while hair is emerging non-invasive biomarkers. Hair is unique character found on all mammals but not on other animals. Hairs may be defined as slender filamentous outgrowths of the skin and are primarily composed of keratin. The present paper highlights the various features of hair which makes it a reliable biomarker such as it is non-invasive, easy to transfer and store, free of contamination, easy sampling and non expensive. It has time detection window of 1 month to a year. However, there are areas of hair research which need further attention.
Keywords: Hair, Human biomonitoring, trace element

Introduction

Human biomonitoring (HBM) can be defined as "the method for assessing human exposure to chemicals or their effects by measuring these chemicals, their metabolites or reaction products in human specimens" (CDC 2005). HBM is a scientific technique that allows us to assess whether and to what extent environmental pollutants enter humans (Alves 2014). The integral part of the technique is selection of an appropriate biomarker. A biomarker is an objective biological measure that can be utilised to assessed health or make a diagnosis of disease. They are considered as markers of exposure, effect and susceptibility and represent event along a theoretical continuum from causal exposure to resulting health outcome (NRC 1987). It may be defines as "any biological index capable of being measured, which is associated with or indicative of a defined biological endpoint such as development and disease stage" (Rockett and Kim, 2005). Tissue and biological fluids of the body routinely used as biomarker for the determination of various trace elements including essential and non-essential are blood, urine, scalp hair, teeth, nails and internal organs. Of these six specimens, urine gives the what body has lost, not what it has retained, teeth are not readily available, while internal organs are available only from autopsies. Hence blood, nails and hair are the only available options. The blood is invasive biomarker while nail and hair are emerging non-invasive biomarkers. However, the focus of the present endeavour is to highlight on the utility of human scalp hair as an indicator or biomarker of chemical accumulation or exposure of the human body. It is important for the researcher in the field of public health and biological anthropology to monitor the influence of various essential and toxic elements in the human health and biology, which has tremendous effect on the growth and development of young children and adult alike. Most vulnerable are pregnant women, lactating mother, infants, children, old age and industrial worker.

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Hair is unique character found on all mammals but not on other animals. Hairs may be defined as slender filamentous outgrowths of the skin and are primarily composed of keratin. It differs from one animal species to another in the basis of length, colour, shape, root appearance and morphological characteristics. There is also a considerable deal of variability in the types of hairs that are found on the body of a particular animal. In humans, hairs are distributed on the head, pubic region, arms, legs, and other body areas (Sen 2010).

Hair Anatomy and Physiology

Structure*:* The hair follicle in the skin and the hair shaft, which is visible on the body surface are two separate structures of hair. The hair shaft is a dead tissue comprised of a cortex, cuticle cells and some cases medulla in the central region. The medulla is the central part of the hair though most hair lacks it (Buffoli et al. 2013). The cortex represents the majority of the hair fiber composition and plays an important role in the physical and mechanical properties of hair, is the peripheral part and is made up of approximately 50 - 60per cent of macrofibrils which consists of microfibrils, also known as intermediate filaments (IF).The fibrils run to form the fiber axis. In between the fibrils a softer material called the matrix is present which grows from the hair follicle. All these filaments and matrix is composed of keratin protein which are held together by -S-S- linkage which are usually is formed between two cystine residues contained in adjacent polypeptide chains and it is this disulfide bond that is mainly responsible for keratin resistance to destruction (Sen 2010). There are type I and type II polypeptides which are encodes by 28 type I genes and 26 type II genes respectively. All together alpha-keratin is encoded by 56 genes (McLean and Moore 2011), which is a low sulphur keratin compared to high sulphur keratin associate protein (KAP) present in the outer cuticle (Nissimov and Das Chaudhuri 2014).The pigment granules containing mainly melanin are also found in the cortical cells. Melanin is synthesized in specialized cells, the melanocytes, located in the hair bulb. The amount, the density and the type of melanin in the melanocytes determines the colour of the hair (Boumba et al. 2006).
The cuticle consists of scale shaped layers and is responsible for much of the mechanical strength of the hair fiber. The cuticle is made up of a number of layers, which varies from one species to another. Typical human hair has six to eight layers of cuticle. Each cuticle cell is generally 0.3 - 0.5 μm thick and 30×40 μm in width and length. Internally the cells consist of three major cytoplasmic lamellar compartments, the A-band, the exocuticle, and the endocuticle which protect the cortex from physical and chemical insult (Nissimov and Das Chaudhuri 2014).
The hair follicle is another important structural part which is fully autonomous skin appendage with its own hormonal control, its own autocrine and paracrine network and its own cycle, appearing as an incredibly complex and stable structure that summarizes the main rules of tissue homeostasis (Bernard 2005). It comprises of the connective tissue sheath, the dermal papilla, the outer root sheath, the inner root sheath, the shaft, the sebaceous gland, the arrector pili muscle and the hair bulb. Each hair follicle is surrounded by a system of capillary blood vessels at the root (Spearman 1977), the chemicals present in serum can theoretically also be found in the hair, making it a suitable matrix to assess level of trace element in the body. As hair grows, nutrients and trace elements are deposited from the blood flow to the hair follicle and hair shaft.

Human Hair Growth: Hairs grow during a phase called *anagen*, and they are eventually shed, only to be replaced by newer ones. When hair is naturally ready to be shed, the follicle becomes inactive during a phase called *catagen*. The follicle then becomes smaller, and becomes detached from the dermal papilla at the base, during the phase called *telogen*. The basal cells in the hair

matrix then produce a new hair follicle during anagen. Hair physiology is rather complex and not very well understood. However, there seems to be agreement that the mean (SD) rate of hair growth of the scalp is generally 1 (0.3) cm per month (Kintz et al. 1992; Zahlsen and Nilsen 1990; Uematsu et al. 1995). Hair growth rate has been calculated as 1.1 cm/month using drug markers incorporated into the hair through the systemic circulation (Miyazawa and Uematsu 1992). The anatomical location of the hair is the most important factor in hair growth rate. Scalp hair grows more quickly than pubic or axillary hair, and 85–90 per cent of it is continuously found in the growing stage. In general, scalp hair grows faster in women than in men (Saitoh et al. 1967) which may be related to female hormones. Other factors such as race and age may also affect hair growth rate, but there is no strong evidence in the literature. Bearing in mind these sources of variability, each cm of scalp hair reflects approximately one month of past exposure (Uematsu et al. 1995; Nissimov and Das Chaudhuri, 2014). The study has shown cm-by-cm distribution of nicotine has been found to approximately match the self report of the month-by-month mean number of cigarettes smoked daily (Uematsu, 1993).

Hair as Biomarker

Advantages of Hair as a Biomarker*:* Most of the argument for hair as biomarkers will clear on the light of biology and structure of human hair. The non-invasive status of hair sampling is explicit in the comparison with the process of blood sampling which involve venepuncture, the act of puncturing the vein for giving a drug or removal of blood. This procedure is without complications, which sometime can be fatal. Complications that can arise from venepuncture include haematoma formation, nerve damage, pain, haemaconcentration, extravasation, iatrogenic anaemia, arterial puncture, petechiae, allergies, fear and phobia, infection, syncope and fainting, excessive bleeding, edema and thrombus (Buowari 2013). In comparison hair sample collection only involve cutting the hair from the occipital region of the head without any pain. In some situation urine collections also involve invasive method and there is always a big chance of contamination, which is negligible in case of hair. For getting urine sample one have to completely rely on instruction given to subject. Both blood and urine are prone to contamination which needs preservatives, sterile and air tide containers.

Depending on the intended laboratory analyses, whole blood and blood fractions may be stored under a variety of conditions. In general, plasma or serum should be stored in mechanical freezers at −80°C and lymphocytes or other cellular specimens should be stored in the vapour phase of liquid nitrogen at −150°C or lower when long-term viability is necessary (Vaught 2006). There is huge variation on temperature maintenance for storage according to study design. Storage of whole blood is futile for more than four days (Heins 1995). Similarly the guidelines for urine testing recommend the use of chemical preservatives if the specimen cannot be processed within 2 hours of collection. Otherwise, these specimens should be refrigerated at 2-8°C. A variety of urine preservatives (tartaric and boric acids being the most common) are available that allow urine to be kept at room temperature while still providing results comparable to those of refrigerated urine. Generally, the length of preservation capacity ranges from 24 to 72 hours.

Again the transportation of this biologically active material needs proper temperature regulation and a robust case/rack to hold tubes or containers upright with sufficient absorbent for spill. All this make the blood, urine and even saliva upto some extent as expensive biomarker. On the other hand hair does not need all this complex storage facility and precautions. Blood is heterogeneous in nature and consists of plasma, serum, leucocytes or erythrocyte. Compare to the complex blood, hair has distinct advantages which is homogeneous and metabolically inert (Sen 1996).

Blood and urine are affected by situation and time of collection such as fasting, morning, before and after meal etc. However, hair is free from situation and time of collection.
It is clear from the preceding discussion the advantage of hair are it is non-invasive, easy to transfer and store, free of contamination, easy sampling and non expensive.

Biological Feature of Hair as Viable Biomarker: The hair matrix cells actively divide in a hair follicle and sensitively reflect the physical conditions of the human body (Terada et al. 2013). Hair has simple matrix, relatively high concentration of trace elements. In addition the ability to measure a large number of, potentially interacting, toxic and biologically essential elements is indispensible (Yang et al. 2015). High element level in hair gives more sensitive and accurate analytical result. Metal-protein complex is formed between the metal deposited and the sulfdryl (-S-S-) linkage of the hair matrix.
Metals can also bind to the hair structure through melanin, which determines the hair colour. Melanins are polyanionic polymers containing negatively charged carboxyl groups and semiquinones at physiological pH and as a result can bind cations by ionic interaction (Larsson 1993). Organic amines and metal ions have a high melanin affinity, because they are positively charged at physiological pH and interact with the melanin polymer by electrostatic forces between their cationic groups and the negative charges in the melanin polymer. The ionic binding can also be enhanced by other forces such as van der Waal's attraction (Larsson 1993). Uncharged metals, e.g. elemental mercury, may also bind to the hydrophobic core of the melanin polymer in the hair structure (Kronstrand et al. 1999).
The various trace or toxic elements enter the hair through matrix, sebum, sweat and epidermis (endogenous sources) and through water, air, dust, oils, lacquer and shampoos (exogenous sources). The model for incorporation of elements in hair is diffusion from blood into cells of hair follical. This is followed by diffusion of body secretion (sebum, sweat) during and after shaft formation. In the same process after the hair shaft formation deposition from external environment takes place (Gill et al. 2004). Hair gives idea of past exposure in case of endogenous deposition and only of present exposure in case of exogenous deposition. Hair provides continues concentration of minerals where as blood and other body fluids give transient concentrations (Yang et al. 2015). As a result hair provides provide past deposition or chronic accumulation of any trace element on the human body. Mineral analysis of hair has become most dependable precise and well recognised technique in clinical investigation with the advent of new technology (Obrusnik 1986).
As previously discussed human scalp hair grows at the rate 1 cm/month using drug markers incorporated into the hair through the systemic circulation. The location of the hair is the most important factor in hair growth rate. Therefore, when using hair samples for retrospective analysing to external substances of interest, it is important to measure the length of the analysed hair segment from the scalp. However, if the aim is to determine the concentration of the element per weight of hair as a measure of usual exposure, regardless of the time of exposure, or if history of past exposure was constant, then the length of hair sample becomes irrelevant. This is not possible with the blood and urine sample. These body fluids have time detection window of an hour to a day only.
There are concerns regarding the sampling procedures as harmonized or sufficient information on the applied sampling procedure is lacking. In a recent report, a harmonized approach was suggested to cut hair near the scalp at the occipital region of the head. This area has less variability in the hair growth rate due to uniform growth pattern and limited per centage of non-growing hair,

less influence from sex or age, and large and constant blood irrigation, thus better translating an internal exposure (Harkey 1993; Tosi and Piraccini 1999; Mangin and Kintz 1999).
The amount of an element that is irreversibly incorporated into growing hair is proportional to the level of the element in other body tissues (Onuwa 2012). Mercinek et al. (2015) has shown significant correlation between dietary calcium and hair level. Study has also found subsequent deficit in the soil and the hair sample analysis (Tommaseo et al. 1998). Therefore, hair analysis may provide an indirect screening test for physiological excess and deficiency of elements in the body. Clinical research indicates that hair levels of specific elements, particularly potentially toxic elements are highly correlated with pathological disorders. All these feature make hair a suitable specimen for epidemiological studies and potentially for diagnostic considerations.

Methods and Techniques

The good laboratory practices and validated methodology will generate precise, accurate, and reliable results (Bass et al. 2001). Determination of trace metals in biological samples requires sensitive and selective techniques. There is great concern in the use of hair cutting instrument from scalp as there is high chance in contamination and use of a quartz blade is suggested (ATSDR 2001). After collection of sample storage in the unsterile plastic bag and container should be avoided. Use of paper bag is suggested by other. Before analysis appropriate washing method should be followed. A reliable washing method should distinguish between the external contamination and internal deposition. Available washing methods are non-ionic detergent (Sen and Das Chaudhuri 2001), 0.1M HCl, 1 per cent (v/v) sodium lauryl sulphate (SLS)/ detergent, (Morton 2002), hexane ethanol method, acetone ether detergent method (Assarlan and Oberleas, 1977; Sen 1996), (0.5%, v/v) Triton X-100 (Razaqui 2008) and de-ionised water treatment. Some prefer not to wash hair sample (Bouchard 2007). Usually samples are digested using reagent like HNO_3, H_2O_2 and heat treatment. Digestion is important to discover the total mineral content of human hair. The International Atomic Energy Agency (IAEA) method for washing hair involves sequential washing with acetone, water and acetone (IAEA 1983).
The most reported techniques for determination of metals in hair are neutron-activation analysis , flame atomic-absorption spectroscopy, electrothermal atomisation atomic-absorption spectroscopy, stripping voltammetry, inductively coupled plasma atomic-emission spectroscopy (ICP–OES) and inductively coupled plasma mass spectrometry ICP–MS (Bouchard 2007; Morton et al. 2002; Moreda-Piñeiro et al., 2007). Other techniques involve proton induced x-ray emission (PIXE) (Jung 2001) and energy dispersive x-ray fluorescence technique (EDXRF) (Onuwa et al. 2012). Important recently develop method involve automated metal-leaching procedure, by pressurized-liquid extraction (PLE), combined with a rapid simultaneous detection system—inductively coupled plasma–optical-emission spectrometry (ICP–OES) (Moreda-Piñeiro et al. 2007).

Future Directions

Human health is continually under threat from the pollution which may pose biological challenges without producing frank disease or clinically recognisable syndromes. Just as suboptimal nutrition may produce reduced growth without nutritional deficiency disease, pollutant burden may produce subclinical effect without producing frank disease. These are magnified by industrial expansion, transfer and mobility of goods, climate change and population growth. The researcher in the field of public health and anthropology needs to keep pace with such environmental change. There are different type of environmental contamination which has direct and indirect influence on human health and development. Hence, there should be more attention to measuring environmental contaminants and human exposure to them.

In this regard hair stands out as viable option and some aspect of it need more focus. The humble effort towards understanding the process and kinetics of substances incorporation in the hair shaft is necessary. Still there is need for correlation study between the level of hair and other biomonitoring agents (blood, urine, target organ sample etc). Numbers of washing methods are available, though there is need to develop the more appropriate hair washing method which can distinguish between endogenous and exogenous deposition. Different laboratories use different methods when conducting hair analysis, there is no standards specify how hair samples should be collected, stored and analyzed. This will mislead the health professionals and researcher. So the study should clearly mention the sampling procedure. This in turn will help to develop appropriate reference level for hair trace elements (Druyan et al. 1998). Appropriate reference values of hair trace element content are required for correct interpretation of biomonitoring data. The studies conducted in this regard are Skalny et al. (2015) and Mikulewicz et al. (2013) and a lot needs to be done.

Conclusion

Given the pervasive role of trace elements in the human biology, its biological monitoring becomes utmost important. Yet, the process is not always easy as researcher in the field is always concern with most reliable and easy biomarker to ascertain the level of trace element in the human body. Trace elements are important and they are two types such as essential and toxic. As name imply essential elements are important for the various life process and metabolic function in human biology. On the other hand there are some heavy elements which are detrimental to health even in minute quantity as they compete with other essential element in the various metabolic pathways. To understand such imbalance role of biomarker is immense. Hair is one such emerging biomarker with a number of reliable and viable features. Though there is need for more studies to highlight on the usefulness of hair as biomarker.

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