Human Scalp Hair As An Indicator of Environmental Lead Pollution and Lead Exposure

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ABSTRACT Today, assessing human exposure to lead has assumed paramount importance. Human scalp hair can be successfully used to document population exposure to lead. Elevated human scalp hair concentrations have been found among the residents of urban/industrial areas over those of rural/agricultural areas. The prevalent methods of determining lead concentrations in human scalp hair and its applications are discussed. In modern day research, trace element analysis of Human Scalp Hair (HSH) bears a lot of potential in environmental, clinical, forensic and archaeological sciences. The following presentation discusses the different aspects of lead pollution, effects of lead on the human body and the role HSH plays in tracing and documenting lead pollution and exposure in human groups.

ENVIRONMENTAL POLLUTION

Environmental pollution may be defined as the release of extraneous substances or energy into the environment by humans that induce unfavourable changes directly or indirectly affecting mankind. These extraneous substances (pollutants) are chemical, physical or biological agents caused by over-population, urbanisation and industrialisation. Schell (1991) defines pollutants as "materials or energy which are unwanted to some degree, presumably because of interference with human biologic well-being". The substances likely to be serious environmental pollutants fulfill all or most of the following four criteria — a) large production b) toxicity c) persistence d) accumulation.

LEAD AS A POLLUTANT

Due to its widespread occurrence and poisonous effects, the toxic element lead (Pb) has been very widely studied in man. Pb has been known to humans from ancient times and, in fact, Pb from poorly-fired pottery (Nriagu, 1983) and indoor air-pollution from ineffectively drafted hearths (Eisenbud, 1978) may be the main sources of Pb during different times. In the developing countries like India, environmental-Pb is a major concern today (Sinclair et al., 1973; Aggarwal et al., 1979; Khandekar et al., 1984; Albert and Badiilo, 1991; Beck, 1992) where a major part of environmental-Pb is due to Pb in gasoline (Caplin et al., 1984; Fergusson et al., 1986). Other sources of environmental-Pb are mining, smelting and painting. There are also specialised occupations like smelting and ceramics that lead to exposures to Pb. Apart from these, the common sources of Pb-exposure in the non-occupational environment are air, food, dust, soil and Pb-based paints (ATSDR, 1988). The health hazards resulting from exposure to environmental-Pb have been very pertinently reviewed by Bushnell and Yaeger (1986), Schell (1991) and Beck (1992). Exposure results primarily due to inhalation and to a lesser degree, due to surface absorption.

MODE OF ACTION AND EFFECTS OF LEAD

There is no known nutritional value of Pb and the element is stored in the bone replacing calcium (De Michele, 1984). Most of the current knowledge on the effects of Pb on the human body come from laboratory experiments on animals (Carpenter and Ferm, 1977; Lorenzo et al., 1978; Penumarthy et al., 1980; Mykkanae et al., 1982; Hirai et al., 1991).
In the cellular level, Pb interferes with the activity of the mitochondrial enzyme delta-aminolevulinic acid synthetase, the cytoplasmic enzyme delta-aminolevulinic acid dehydrase and the intramitochondrial enzyme ferrochelatase. Pb alters cell membrane structure and membrane ion function (Apostoli et al., 1988; Bertoni and Sprengle, 1988). This element is also a potent inhibitor of the Na-K ATPase (Raghavan et al., 1981; Bertoni and Sprengle, 1988). It has been suggested by Webb et al. (1981) and Weiler et al. (1988) that this inhibition of Na-KPase could be involved in some health disorders induced by Pb. It has also been reported that Na-KPase inhibition contributes to the signs of Pb poisoning, including cerebral edema and hemolysis (Raghavan et al., 1981; Bertoni and Sprengle, 1988).

Exposure to Pb leads to impaired reproduction and reduction in both prenatal and postnatal growth in animals (Der et al., 1974; Bell and Thomas, 1980). Interestingly, though some human studies portrait an increased risk of prematurity at low Pb-levels (Fahim et al., 1976; Huel et al., 1981), others failed to do so (Gershnik et al., 1974; Wiberley et al., 1977). Studies have also indicated that Pb-exposure and birth weight is related in humans (Moore et al., 1989; Bornschien et al., 1989) with even focuses being at risk from maternal environmental-Pb exposure (Black, 1988). Children are the most affected by Pb and they store Pb at a faster rate than adults (Duggan, 1983; ATSDR, 1988). There are studies showing evidences that Pb-exposure causes adverse neuropsychological effects like hyperactivity, attention and learning reduction, and perceptive and motor function impairment in young children (Cooney et al., 1987; Huel et al., 1992). Studies have also established negative effects of Pb on postnatal growth (Lauwers et al., 1986; Little et al., 1989; Shukla et al., 1989; Frisancho and Ryan, 1991). High Pb-exposure over a long period of time leads to weakness, paresthesia, neurological and endocrinol dysfunction, anemia and finally death (Rohn et al., 1982; Seigel et al., 1989; Rae, 1991).

**UTILITY OF HAIR STUDY FOR THE ANALYSIS OF LEAD**

The tissue specimens of the body that can be utilised for the determination of Pb concentration are blood, urine, scalp hair, teeth, nails and internal organs. Of these six specimens, urine gives information on what the body has lost, not what is retained, teeth are not readily available, very little are known about nails while internal organs are available only from autopsies. Hence, blood and hair are the only two viable options the researcher is left with.

As hair bind Pb at high concentrations and its growth is continuous, it gives a record of long-term exposure. On the other hand, blood Pb gives a measure of Pb level only at that point of time (Chattopadhyay et al., 1977; Laker, 1982; Limic and Valkovic, 1986; Rendic and Valkovic, 1988; Radomska et al., 1991a, 1991b). Reservations have also been expressed about the measurement of concentrations of different elements like Pb in the blood due to its sensitivity to numerous other influences (Aggett, 1979). Blood is heavily buffered against significant change in the concentration of certain elements such as calcium (Hendrickson et al., 1969). Furthermore, blood is complex and heterogeneous in composition where elemental concentrations may be measured in whole blood, plasma, serum, leucocytes or erythrocytes. Use of hair has distinct advantages as unlike blood, it is homogeneous and metabolically inert. This is further strengthened by the fact that the levels of elements are between 50-100 times higher than the levels of the same in blood or urine (Hansen, 1981; Laker, 1982; Attar et al., 1990; Valkovic, 1992). This makes the analytical procedure simpler.
Above all, hair can be readily procured by a non-invasive technique, easily stored and transported. Blood analysis, on the other hand, requires venepuncture and transportation of a biologically-active material (Wilhelm et al., 1989, 1990; Attar et al., 1990; Valkovic, 1992). The advantages of hair over blood have been further discussed and elaborated upon by a number of researchers (Lazar, 1974; Rabinowitz et al., 1974; Fletcher, 1982; Hambidge, 1982; Meister, 1982; Manson and Zlotkin, 1985; Taylor, 1986; Evans and Jervis, 1987; Klevay et al., 1987; Chatt and Katz, 1988; Katz and Katz, 1992).

**NATURE OF METAL BINDING IN HAIR**

Hair is composed of keratins, which are traditionally identified as cystine-containing proteins. These proteins form two large groups — i) Intermediate filament proteins, and ii) Intermediate filament associated proteins (Powell et al., 1991). These two groups are further subdivided into a number of families (Powell and Rogers, 1990) with each keratin family containing several genes. Many of these genes have been isolated and sequenced (Powell and Beltrame, 1994). Of the several types of bonds that stabilize the keratin molecule, the most important is -S-S (sulfdryl). It is generally accepted that the trace elements are bound to the sulfdryl groups (Hopps, 1977; Chittleborough, 1980). Trace elements combine with these sulfdryl groups to form metallo-proteins. The formation of keratin from these metallo-proteins in the matrix cells results in the incorporation of elements in the hair structure. However, in their study on adsorption and elution of metals in hair, Mikasa et al., (1988) put forward an alternative suggestion that probably its the carboxyl group (not the sulfdryl group) that are the binding sites for metals. Elements such as Pb, enter the hair via matrix, sebum, sweat and epidermis (endogenous sources), and via water, air, dust, oils, lacquer, and shampoos (exogenous sources).

**METHOD OF DETERMINATION OF HAIR LEAD**

Prior to the determination of the element concentration, the hair samples are washed to remove superficial contamination. A problem often encountered here is that these washings remove trace elements from the hair to some extent. The two factors responsible for this are the nature of the washing agent and time of contact. Ideally both these factors must be so selected that further washings do not change the elemental concentrations (Valkovic, 1992). At present, there are three prevalent methods of hair sample washing:

a) using a detergent (Bergomi et al., 1985; Jones et al., 1987; Hajem et al., 1990; Gonzalez-Reimers et al., 1991)

b) using organic solvents (Bate and Dyer, 1965; Chattopadhyay et al., 1977; Wilhelm et al., 1991, 1994)

c) a combination of the above two (Petering et al., 1973; Jamall and Jaffer, 1987).

Since Pb is in the particulate form, a successive washing method using organic solvents to remove the oil, lacquer and particulate matter, an anionic detergent to remove the absorbed heavy metals, and distilled water and acetone has been put forward by Petering et al. (1973), and later used by Jamall and Jaffer (1987) and Jamall and Allen (1990). In their study, Clarke and Wilson (1974) concluded that the use ethyldiaminetetraacetic acid (EDTA) as a washing medium was sufficient to remove surface contamination for hair samples for Pb analysis. Later on, Raghupathy et al. (1988) also confirmed that EDTA seemed to be the most suitable washing medium. On the other hand, Chittleborough (1980) preferred a "no-wash" approach, while a decade later Schuhmacher et al. (1991) advocated a non-ionic detergent.
wash method. Hence, there is still no universally-accepted method of hair washing and controversy continues to brew. Till this vital aspect is solved, comparing results of different studies can be vindicated by different washing methods.

After washing and drying, the samples are either wet-digested or dry-ashed. Wet-digestion is done by using a mixture of nitric acid and hydrogen peroxide, or nitric and perchloric acids, or nitric and sulfuric acids. Dry-ashing is done at very high temperatures and the ash obtained dissolved in nitric acid. However, a majority of the researchers prefer to use the wet-digestion method (Piccinini et al., 1986; Bache et al., 1991; Sukumar and Subramanian, 1992a, 1992b, 1992c).

The Pb concentration in the digested samples can then be estimated using flame atomic absorption spectrophotometry (Petering et al., 1973; Boiteau et al., 1983; Piccinini et al., 1986; Wilhelm et al., 1989, 1990, 1991, 1994; Ahmed and El-Mubarak, 1990a, 1990b; Jamall and Allen, 1990; Gonzalez-Reimers et al., 1991; Schuhmacher et al., 1991; Sukumar and Subramanian, 1992a,b,c). Apart from atomic absorption spectrophotometry, there are other techniques like neutron activation analysis (Bate and Dyer, 1965), conventional stripping voltammetry (Bache et al., 1991), mass spectrometry (Rabinowitz et al., 1974), instrumental photon activation (Chattopadhyay et al., 1977) and inductively-coupled argon plasma spectrometry (Ahmed and El-Mubarak, 1990a, 1990b) that can be used for the determination of Pb in the digested samples.

HAIR ANALYSIS FOR DOCUMENTING ENVIRONMENTAL EXPOSURE TO LEAD

In the context of present day research, monitoring of human exposure to environmental-Pb bear utmost importance (Benccko et al., 1989; Cikrt and Bencko, 1990). It is now well established now that determination - HSH Pb concentration is a successful way to screen populations for exposure to environmental-Pb. Elevated levels have been obtained among resident of industrial/urban areas over agricultural/rural areas.

It was way back in the 1970s when an American study (Hammer et al., 1971) estimated the mean HSH Pb concentration among the residents of a rural area to be 8.2 μg/g while that among the residents of an industrial area to be 80.2 μg/g. Three years later Lagerwerff and Brower (1974) showed mean HSH Pb levels to be 26.0 μg/g in a low Pb-exposure area, compared to a mean of 36.0 μg/g in a high Pb-exposure area. This trend was further established by Chattopadhyay et al. (1977) who in a Canadian study found mean HSH Pb concentrations to be 10.1 μg/g in a rural area, rising to 16.9 μg/g in an urban area and eventually reaching a very high value of 45.2 μg/g when individuals near a Pb-smelter were sampled. Results of a study in Brazil by Carvalho et al. (1984) showed that Pb concentration in HSH increased from the rural zone to the urban zone. A significantly higher mean HSH Pb value was obtained among the residents of an urban area (25.11 μg/g) over that of a rural area (15.3 μg/g) in Italy by Bergomi et al. (1985). The same picture was revealed by Jones et al. (1987) in their study in Papua New Guinea where the mean HSH Pb concentrations in a remote village and in a town were found to be 23.2 μg/g and 36.3 μg/g respectively. A study in Germany (Wilhelm et al., 1989) came up with a high urban HSH Pb mean value of 3.8 μg/g and a low rural mean value of 1.4 μg/g. A maximum HSH mean concentration of <10 μg/g was reported by Bertillo and Bonard (1989) from a rural area in Argentina. The same study found a maximum mean HSH urban concentration of <20 μg/g. Jamall and Allen (1990) reported high Pb concentrations in the scalp hair of persons residing in the city of Karachi.
HUMAN SCALP HAIR AS AN INDICATOR

(mean: 31.7 µg/g) and a low Pb concentration from the residents of a village in Bangladesh (mean: 5.0 µg/g). A Spanish study by Schuhmacher et al. (1991) too came up with the same results of a high HSH Pb concentration in an industrial area over that of a rural area (mean 9.38 µg/g versus mean 7.80 µg/g).

All the above-mentioned studies and those of Creason et al. (1975), Bogen et al. (1976), Baker et al. (1977), Milosevic et al. (1980), Garcia et al. (1983), Limic and Valkovic (1986), Wibbowo et al. (1986), Prucha (1987), Moon et al. (1988), Ahmed et al. (1989), Bergomi et al. (1989), Lepera et al. (1989), Traktenberg and Lukovenko (1990), Wilhelm et al. (1991, 1994), Radomska et al. (1991a, 1991b), Foo et al. (1993) and Revich et al. (1994) confirm than indeed HSH is in a position to successfully document population exposure to Pb. This has very important implications as environmental-Pb is a major health concern. As a result, many agencies and countries all over the world have elaborate research projects for the biomonitoring of environmental-Pb exposure. Among them are a UNEP/WHO study (Friberg and Vahter, 1983) and two IAEA studies (TECHDOC-330, 1985; NAHRES, 1993). However, Indian studies involving trace elements in HSH are only a handful in this regard, and includes those of Raghupathy and Sharma (1985), Sukumar and Subramanian (1992a, 1992b, 1992c), Chatterjee et al. (1993) and Sinha et al. (1993). With such a dearth of studies, there is scope for ample research in this area in India.

What does high levels of HSH Pb concentrations indicate? It has been shown by Krause and Chutsky (1987) that hair Pb concentrations of >20 µg/g is to be considered a cut-off between high degree of Pb-exposure and low degree of Pb-exposure. So, once a population is screened for Pb-exposure, those individuals having a HSH level of >20 µg/g fall in the high risk group. Once this high risk group is isolated, only then blood samples from these individuals should be analysed for Pb concentrations. Adult inorganic-Pb intoxication is defined as whole blood Pb levels of >38 µg/dL (Rempel, 1989). A chelation therapy of intravenous calcium-EDTA is the method of treatment for individuals affected by Pb intoxication (Grimsley and Adams-Mount, 1994).

The above presentation deals at length on the toxic effects of Pb on the human body and the role that HSH can play in documenting and screening populations exposed to Pb. The utility of HSH over blood in this regard has been aptly summarised. A major fact is that researchers are now in a position to use scalp hair to screen populations for Pb-exposure. Only when the high-risk group is identified, the need to estimate blood Pb arises. This field is challenging field nowadays, and a lot of thrust is being given in this area. This is a field which holds high promise and expectations in a developing country such as India.

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HUMAN SCALP HAIR AS AN INDICATOR

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