Scalp hair as a screening tool for detecting trace elements concentration

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Abstract
Health issues associated with critical trace elements that arise from insufficient (i.e. poor intake) and over-exposure. Measuring sensitivity is also a major problem for geneticists. Among noninvasive assays designed to quantify long-standing exposure to critical trace elements, scalp hair could be the most theoretically biological network. This analysis collects recent information relating to the safety of the scalp as a critical predictor of sensitivity to trace minerals such as cobalt, copper, boron, manganese, iron, selenium, molybdenum, silicon, zinc and vanadium. Particular consideration has been given to the exposure period through which the hair of the scalp is represented, and to the human variation of exposure rates over time in this matrix and the association between scalp hair and other essential markers, human characteristics and environmental influences. Where conventional and contemporary methods of washing and digesting scalp samples have been tested to train models for the measuring of trace metals in the scalp hair. The emergence of different approaches and new analytical procedures has made the usage of other less intrusive or noninvasive matrixes, for example hair. The existence of a chemical in these matrices indicates toxicity; however, associations between blood and the levels of non-invasive media must be established in order to insure that these quantities are associated to the total body load. The development of unique biomarkers which can be tested in this matrix would enhance non-invasive bio monitoring. Common spectral techniques such as inductively coupled plasma mass spectrometry "ICP-MS", plasma spectroscopy laser ablation "LA-ICP-MS", atomic absorption spectroscopy, etc. They have very good analytical performance, but the samples in them are subject to significant damage or are destroyed in addition to being environmentally hazardous because they require the use of hazardous chemicals and take a relatively long time. Compared to the laser induced breakdown spectroscopy "LIBS" process we find that this process has several advantages, such as the flexibility and smallness of the device and the convenience of sample preparation, as well as the ability to analyze samples from a distance, particularly in hazardous and inaccessible places.

Keywords: trace elements, scalp hair, spectral techniques, ICP-MS
1. Introduction

In 2000, Kolmogorov et al. conducted many epidemiological trials to prove that exposure to toxic elements would contribute to a carcinogenic impact on humans and animals (1). The following year, Kolmogorov et al. noticed that necessary trace metals / metalloids are either required to sustain critical physiological functions or are part of biological systems of crucial human functions (2). Esteban et al in 2009 obtained samples of human hair for the purpose of examining trace elements due to their inclusion is a biological resource that is quickly and non-invasively extracted at low expense and conveniently preserved and transported to the laboratory for examination (3). In 2005, Chojnacka et al carried out a detailed analysis on hair weaknesses as a biomarker of metal toxicity. It is well established that these components enter the human body in a number of areas, including the respiratory tract, the digestive system and, in certain instances, via the skin via different outlets (water, air, drugs and food). This is then distributed and transported through blood to organs such as the heart, lungs, and removed from the body through various paths: sweat, nails, scalp hair, faeces, urine, and saliva, as seen in Figure 1 (4). Trace components are present in the body tissue at a relatively small concentration, varying from (µg to mg), and are important for some biochemical procedures (5).

In 1990, Kondo et al researched the composition of human hair and found that Scalp hair is characterized as a fibrous skin-derived substance and is distinct from other tissues (6). Although recent studies have related the composition of human hair scalp to its capacity to maintain trace elements in order to explain how scalp hair keeps metals, it’s essential to know the origin of the scalp hair and how the protein of the hair is synthesized and binds the metals together. The scalp hair shaft is a filament of the cell matrix at the bottom of the hair follicle deep within the epidermal epithelium; each follicle includes both glandular and muscle components as seen in Figure 2. Human hair is made up of 15% water, 80% protein and a small amount of an inorganic material and lipids. The mineral content of the scalp hair at a dry ash stage is 0.25-0.95% (7),(8).
2. Trace Elements in Scalp Hair:

Wang et al in 2009 observed that scalp hair has a rare ability to disclose historical details on the dietary state and treatment of specimens (9). Throughout 2010, the scientist achieved an extensive and reliable study of the trace components in the scalp hair for foreign exposure. Hair has also been described as a useful measure of environmental contamination. Heavy metals are introduced into the scalp hair during the growing cycle and represent the presence of these metals in the plasma at the moment of development(10). Skalny et al noticed in 2015 that hair is quickly harvested and does not need any special storage or protection. In addition, some foreign elements are present in the hair at a rate at least 10 times higher than in blood serum or urine(11). In 2016, Baker et al were able to develop a near connection between trace elements and those with breast cancer (12). In the work that has been done, it is reported that hair tissue has a range of advantages over blood and urine, containing: noninvasively substances and samples can be readily collected; is likely to be a long term growth substance; and many trace metals that persist in scalp hair tissue over a span of two to eighteen months. These benefits may offer suitable evidence to determine an individual’s wellbeing over a lengthy period of time, since tissues stay isolated from other physiological processes in human body. The quantities of heavy elements in human scalp hair are the normal concentrations of trace components of hair scalp with a variety of previous research in Table 1.(13)(14)(3).

Table 1: Registered natural or control amounts of some trace elements in hair washed.

| Element | Country          | Concentration (mg/Kg) | n Samples of scalp hair |
|---------|------------------|-----------------------|-------------------------|
| B       | Sweden           | 0.14 – 3.40          | 115                     |
|         | Rio de Janeiro  | 2.0 – 3.0             | 86                      |
|         | Sweden           | 0.34 – 0.90          | 93                      |
|         | 0.006 – 0.135    |                       |
| V       | Rio de Janeiro  | 7.0 – 16              | 84                      |
|         | Sweden           | 5.9 – 33              | 115                     |
|         | USA              | 13.2± 7.2             | 18                      |
| Fe      | Bangladesh       | 16.54 – 314.49        | 45                      |
|         | Rio de Janeiro  | 14 – 36               | 84                      |
|         | India            | 45.4 ± 7              | 114                     |
|         | Sweden           | 8.6 – 97              | 115                     |
|         | Italy            | 7.59 ± 3.42           | 19                      |
|         | Bangladesh       | 4.3 ± 6.29            | 44                      |
|         | Pakistan         | 16 – 22.8             | 152                     |
| Cu      | Bangladesh       | 126 – 166             | 84                      |
|         | Sweden           | 67 – 199              | 124                     |
|         | India            | 193.7 ± 34            | 123                     |
|         | Pakistan         | 237.2 – 263.8         | 152                     |
| Zn      | Italy            | 132 ± 48              | 28                      |
3. Washing Procedures:
The key purpose of the washing process is to eliminate external pollutants from the surface of scalp hair in order to have a true level of endogenous metals. The analyses of previous and existing research have indicated that there are several methods used in hair washing processes and the most significant of these methods used in hair washing. Several procedures for the washing of scalp hair samples were proposed in the literature. The several scholars state that an ideal washing process would only remove exogenous elements and leave endogenous metals in place. The literature review concluded that there were three washing procedures to determine influence of scalp hair washing. Relative to the unwashed part, i.e. sequential washing in the ultrasonic bath with: (i) Method A: CH₃COCH₃-H₂O-H₂O-CH₃COCH₃; (ii) Method B: ether-Triton X-100-H₂O-H₂O; and (iii) Method C: ether-CH₃COCH₃-H₂O-ether (15),(16). For the production of the process, the hair sample was washed using such procedures as may be used as the correct tool for the analysis of hair. The method A has proven successful in removing external trace elements and preserving internal trace elements, this method has become accredited by the International Atomic Energy Agency.

4. Digestion Methods:
In 2008, Kazi et al. examined digestion techniques being used to dissolve the organic material of the sample, leaving just the inorganic trace behind. In this analysis, three procedures of digestion have been used to digest hair sample, which include: (1) Method A: dry ashing without the need for HNO₃; (2) Method B: dry ashing of pre- HNO₃ digestion in a steam bath in a smoke fridge; and (3) Method C: wet digestion using HNO₃ in a tube. In dry ash, the material is gradually decomposed in a muffle furnace over a ramped temperature from (200 to 500°C), leaving behind an inorganic trace that is soluble in aqueous nitric acid. In the case of wet digestion, oxidizing acid, such as HNO₃, can destroy biological matter.(17).

4.1 Dry ashing (Method A)
Dombovári & Papp used a procedure in 1998 to weigh (0.50 ± 0.01 g) of unwashed hair in a clean - dried 100 ml beaker (10 replicates), dry ash for two hour at 200°C in a muffle furnace, before increasing the temperature to 500°C overnight. The ash was dissolved in 1L of distilled HNO₃ and then diluted to 50L with deionized H₂O in a polyethylene volumetric flask. The hair samples were centrifuged at 3000 rpm for ten minutes and then screened via the Millex filter (0.45 μm). Samples were then moved to 50 ml of Sterilin labelled Centrifugal tube and packed in the refrigerator at 4°C (18).

4.2 Dry ashing - pre-HNO₃ (Method B)
Salnikova et al used the same mass of hair in 2018 using this process (0.50 ± 0.01 gram) and then one ml of concentrated HNO₃ was added to each sample (10 replicates) using a beaker in
a smoke cupboard and left overnight. The same measurements (as defined in Process A) were used to digest scalp hair samples using a muffle furnace(19).

4.3 Wet digestion (Method C)
In 2017, Skalny et al used the same weight (0.50 ± 0.01) gram of samples and moved to a clean-dry tube for process production. 1ml of HNO₃ was added to each tube, and the digestion tubes were placed on a heating block at 170°C until the sample hair was digested, i.e. half hour. All digested scalp hair was diluted with deionized water using a polyethylene volumetric flask, resulting in a dilution factor of 100 fold dilution rate. Sample hair solutions were centrifuged at 3000 rpm for ten minutes and filtered through a Millex filter. Digested Sample solutions have been stored in a 50 ml centrifugal tube labelled and stored in the refrigerator at 4°C(20).

In the year 2020 a method for digesting hair models was adopted, which includes two steps: (i) Digestion approximately 0.2 gram of pre-cleaned scalp hair models were placed in 60 ml of Teflon containers and then 5 ml of highly pure Nitric acid was added. One sample was heated on a hot plate at 70°C overnight and the other samples were at 160°C overnight. So as to explore the impact of hydrogen peroxide on recovery levels, 0.2 ml of hydrogen peroxide is applied to the samples hair and interacted for 30 minutes. Samples scalp hair are dried and dissolved in 10 ml of 5% Nitric acid. (ii) In the digestion process involves two aliquots of methods (i) and (ii) dried and then furthermore responded with 10 ml of a pure Nitric acid in Teflon containers. Every sample hair was digested on a hot plate at 70°C and at 160°C for 8 hours. The samples hair were then washed and dissolved at 10 ml of 5% Nitric acid (21).

Method C has proven its efficacy over other methods, which is the method that has been adopted by the International Atomic Energy Agency (IAEA).

5. Spectroscopic techniques, basics:
The former is the most often techniques used for studying scalp hair involved X-ray fluorescence (XRF), inductively coupled plasma optical emission spectroscopy (ICP-OES), atomic absorption spectrometry (AAS), graphite furnace atomic absorption spectrometry (GFAAS) and Isotopic dilution mass spectrometry (IDMS). Since mid-90s, inductively coupled plasma mass spectrometry (ICP-MS) has been utilized by most modern clinical laboratories. Kumakli et al in 2017 using ICP-MS had the benefit of noticing trace metal amounts at lower rates than its predecessor ICP-OES, which is useful for the study of toxic metals detected at amounts as small as part per billion (ppb)(22). Pozebon et al in 2017 and Scassillati et al in 2020 concluded that ICP-MS technology is characterized by the following characteristics: rapid quantitative and semi-quantitative analysis of trace elements, multi-element technique, solid and liquid sample, Only small sample quantities needed (usually-3-5 ml for solution), comprehensive analytical range (ppt-ppm for solution), high sensitivity, high precision and accuracy, and isotope ratio measurements which render it distinct and preferred over previous techniques are possible (23)(24).

The most recent spectroscopic studies included interaction of electromagnetic radiation with matter, and therefore there are at least 50 types of spectroscopic techniques classified by type of radiation Uv-Vis, X-ray, IR and so on, which can also be classified by type of interaction such as absorption spectroscopy, emission spectroscopy, etc. In addition to certain special types of spectroscopic technique, such as Raman, which is based on inelastic scattering and inductive plasma coupled, with laser ablation in addition to solution nebulisation and other instrumental procedures, various types of tools are offered (25)(22) (26)(27). Table 2 below indicates the most significant variations between the atomic research instruments and the
explanations for their application. Recently, LIBS laser-induced breakdown spectroscopy was utilized for its advantages relative to other forms of study. This method is distinguished by a clear and compact experimental layout and ease of processing samples, as the examination surface of the sample would be less dangerous to the specimen, as well as the opportunity to analyze samples from a distance in unsafe environments that are challenging to access with other techniques. The downside of this strategy is that the measurement limit has a strong and repeated effect which shows that the outcome is unreliable.

Table 2: A general comparison between the performances of different atomic spectrum devices

| The property                  | ICP-MS | ICP-OES | GFAAS | FAAS   | Arc AES  |
|-------------------------------|--------|---------|-------|--------|---------|
| Sensitivity                   | very high | high   | high  | Medium | Relatively low |
| Speed                         | very high | high   | Low   | good   | Low     |
| Accuracy                      | high   | high   | Medium | high   | Medium  |
| Chemical interferences        | Very few | few    | high  | few    | few     |
| Spectral interferences        | not available | few   | high  | few    | Medium  |
| Effect of dissolved salts     | High   | Relatively few | Relatively few | High   | Not affected |
| The required sample size      | few    | Medium  | Very low | High   | solid sample |
| Isotope analysis              | possible | impossible | impossible | impossible | impossible |
| The need for experience       | Important | Important | Important | Simple | Important |
| The price for the device      | very high | high   | Medium | Below Medium | Medium |

6. Results and Discussion

In 2017, Gang Liang et al experimented on human hair in various samples in food and age groups were obtained in Beijing, China. Metals involved (Hg, Cr, Cd, As, and Pb) were examined were further measured in relation to, age, dietary intake and gender. The findings revealed the highest amount of metals detected by Pb, with an average concentration of 1,560 ± 0.780 mg / kg, accompanied by As (0.127 ± 0.078), Cd (0.071 ± 0.032), Cr (0.782 ± 0.394), Hg (0.284 ± 0.094). Trace elements concentrations differed significantly across various age classes, and higher concentrations of As, Pb, Cd and Cr occurred in feminine scalp hair, although greater concentrations of Hg were seen in male scalp hair, indicating that gender and age were not important variables in determining metal concentrations in human hair (28).

In 2016, Baker et al examined the impact of arsenic on patients with breast cancer; the researchers were specifically notified about all research protocols prior to ratification the recognizant consent document. Commonly, participants were questioned at sampling time to collect some general knowledge about their healthiness condition, usual diet and smoking activity. A total of sixty five samples scalp hair were collected from healthy (n=32) and breast cancer patients (n=33) in relation to different forms of smoking (active and none). In other words, high levels of As was found in hair of smokers (16578.40 ± 4254.70 µg/kg) when compared with those of nonsmokers (6541.54 ± 2826.70 µg/kg). The analytical recovery
value for arsenic calculated is 98% (12). This enabled the analysis of any major variations and whether there was a potential association between the amounts of arsenic in such media. Pozebon et al in 2017, in their study, the basic examination of hair is checked in their papers written over the last ten years. They started with a general overview of nature, anatomy and future methods for elementary analysis, where (ICP-MS) was specifically method contributed to quantitative ultra-trace elementary analysis. Importance was provided on quality control, sampling, sample decomposition and washing procedures with comprehensive guidelines summarized in the tables in addition to the use of scalp hair to classify sex, illnesses, medical environments, nutritional status and contamination sites.(23).

Data on the isotope level, chemical speciation analysis and computational pre-concentration are often known for scalp hair. Ultimately, the capacity of (LA-ICP-MS) to provide spatial time-tracking and precision for the measurement of scalp hair strand components instead of traditional bulk processing was established as a possible future development in the sector. Their dissertation was an significant cornerstone for those who wish to research the topic of heavy elements in human hair. For the first time, X-Ray Fluorescence Spectrometry was used to test the substance that irradiated As and Hg for Penang by Khalid Saleh et al in 2015 to a specified amount of Hg and As in hair from a designated population. This research would be a guide for potential studies to evaluate the human hair ratio of Hg and As for citizens in Penang(29).

In 2005 in Vienna, Stadlbauer et al studied forensic hair from root to tip in order to achieve time-limited testing of trace elements in scalp-hair toxicity. The samples were evaluated to study ratio of concentrations in the inner and outer parts of the scalp hair using (LA-ICP-MS). The findings indicate an aggregation of heavy element from the bloodstream during scalp hair development rather than the products secreted by sweat and sebaceous glands that are accumulated on the surface of the scalp hair. The disparity in the heavy metal amounts of the outer and inner areas of the scalp hair must be understood in terms of the variation between the composition and functioning of the cuticle and the cortex (30).

7. Conclusion

This analysis offered proof of the function of hair as biological matrices for exposure to toxic metals. For certain minerals, there is evidence that scalp mineral concentrations may affect long-term external exposure and repeatable amounts, but this knowledge is undisclosed to certain components. There is also a great deal of confusion regarding the possible position of socio-economic and lifestyle influences and other variables influencing the mineral levels of hair. Some levels of heavy metals may affect exposure to certain nutrients or foods from diets or supplements and may be changed by exposure to smoking. The minerals examined may be influenced by environmental features such as water or soil contamination and accumulation of minerals, while records are scarce for other heavy metals. Standardization of quality management, sample selection, reporting procedures and analytical techniques that improve study and a clearer understanding of the consequences for key levels of this improve and promising matrix the usage of scalp hair in epidemiological study .The advancement and enhancement of analytical methods has less measurement thresholds, requiring further chemical quantities to be assessed. A clear model of such diagnostic strategies is (ICP-MS), which allows the exposure of nano gram of several scalp hair components. Recently, laser-induced breakdown spectroscopy (LIBS) was used for its advantages relative to other forms of study. Quantities of these small concentrations suggest very stringent laboratory requirements owing to the possibility of pollution of the sample. There are several methods used in chemical purposes that have different properties and must therefore be selected according to the framework used, the form of studies and the predicted chemical conditions.
We conclude from this that choosing the appropriate technique for analyzing trace metals in the samples in addition to the importance of using the appropriate washing method as well as using a proper digestion method that does not lead to a loss of concentrations of these elements within the hair scalp.

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