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Research Article

Study of the Hair Matrix in EDX for the Detection of Heavy Metals in Patients with Cancer and Belonging to the Campania Geographical Area

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Abstract

Background: The aim of this study is find a correlation between Heavy Metals presence in Hair matrix and cancer. We have made an analysis of the hair matrix throught the technique of EDX in SEM. The sample collection procedure is not invasive, it allows easy retrieval of the sample with a significant compliance for the patient and the transport and storage do not require special precautions.

Material and Methods: Given the biological characteristics of the sample (hair matrix), we opted for a technique that in our opinion can give our study a high impact in terms of sensitivity, specificity and repeatability of the examination on the same sample. Therefore we have identified in the EDX technique, thanks to its sensitivity in tracking and verifying quantities, very small in terms of nanomoles / nm, of all the elements of the periodic table, including the heavy metals of our interest.

Results: The study investigated a total of 15 patients all suffering from various types of Tumor and all belonging to the same geographical area, with particular reference to the Campania region, enclosed in the municipality of Quarto. Biological samples, human hair, collected in the manner described above, from patients in the city of Quarto were analyzed with EDX method.

Conclusion: In our Opinion this study is the first step for an deeper and widely investigation about Heavy metal and their effect on people that live in That Region of Italy (Campania) especially in some areas that are more interested by that phenomena of pollution of the soil.

Key Words: cancer; EDX analysis; pollution; heavy metals; thallium pollution; molibdenum

Introduction

The choice to carry out a study, by performing the analysis on the hair matrix, allows us to have a greater time window to detect the presence of a certain substance. In fact, if a substance (for example a metabolite) is detectable for a few hours in the blood and a few days in the urine, the same substance is detectable in the hair for a few months or years, depending on its length. Knowing that each hair bulb has its own life cycle, consisting of: a first phase of growth called anagen (the only stage in which the incorporation of drugs would take place) lasting from 2 to 7 years, an intermediate stage called catagenic about 2 weeks and a stage of rest called telogenic of 3-4 months, starting from the proximal part of the hair, that is, close to the skin, it is possible to detect an exposure temporally close to intake, while moving in the distal part, towards the tip , there is an exposure further back in time. In addition, since the growth rate of the hair in the anagenic phase is about 1 cm / month, the segmental analysis per cm of hair can provide information regarding the history and type of consumption of a substance in each of the months corresponding to the segment analyzed [1].

The sample collection procedure is not invasive, it allows easy retrieval of the sample with a significant compliance for the patient and the transport and storage do not require special precautions. Unlike what happens for other biological samples, when analyzing a hair sample and interpreting the result, it is necessary to take into account some peculiarities of the hair matrix, such as its nature (solid matrix), the heterogeneity, any hygienic and cosmetic treatments that could alter the presence and concentration of the substance to be researched. Given the biological characteristics of the sample (hair matrix), we opted for a technique that in our opinion can give our study a high impact in terms of sensitivity, specificity and repeatability of the examination on the same sample. Therefore we have identified in the EDX technique, thanks to its sensitivity in tracking and verifying quantities, very small in terms of nanomoles / nm, of all the elements of the periodic table, including the heavy metals of our interest. Unlike the other techniques, EDX gives us the possibility to investigate the whole range of heavy metals present at the same time (characteristic of this analysis) therefore there is no need for a request from the clinician on the basis of a rationale, being a broad spectrum analysis allows us to investigate small quantities of material with a significant saving in terms of cost of the materials used and with a significant reduction of waste substances coming from the processing of the sample.

The study plans to carry out a survey on the resident population in the Campania region, in particular we will study some municipalities of the "Terra dei Fuochi" (land of fires) [2,12], this will allow us not only to verify the average concentrations of heavy metals in this population, but will guarantee the possibility also to evaluate a parallelism between these heavy metals and the presence of tumors in the subjects investigated [3]. To this end, in fact, subjects with various types of tumors not yet undergoing pharmacological treatment, and they live in the geographical areas will be taken into consideration.

The results lead to the first step in the creation of a standardized method for the analysis of heavy metals on hair in Caucasian subjects with and without cancer. This method becomes not only diagnostic, but it's candidate also as a predictive testing.

Materials and methods

Withdrawal method

The collection of the sample is carried out by medical personnel who explain and carry out the procedure for taking the sample from the subject undergoing the control, fill in the sampling report, have the informed consent signed and fill in the appropriate sections of the chain of custody form. It is essential to prepare Standard Operating Procedures (SOPs) relating to the collection and storage of the sample (1).

These procedures must be strictly followed. We have documented by filling in the appropriate forms:

• respect for the privacy and security of the subject under control at the time of collection;
• verification of the identity of the subject subject to control;
• the correct attribution of the sample to the subject to control;
• that no falsification or tampering of the sample has taken place;
• that no unauthorized access to the sample has taken place;
• the informed consent has been signed by the subject to control;
• the use by the subject under control of particular medicines that may interfere with the analytical results;
• the traceability of the sample through appropriate records of its movements, from the place of collection to its reception in the Laboratory, including the identity records of the personnel authorized to handle it.

According to the quality and safety regulations in force at ISO 9001/2015.

The sample is usually taken from the area corresponding to the back of the head (vertex), as close as possible to the scalp; in fact, it is considered that this region of the head is associated with a minimum inter-individual variability in the speed of hair growth. When the sample is taken on a child or on people with evident hair thinning, the sample collection can be done by taking smaller strands in different areas of the head, but concentrating in the area of the posterior vertex of the head.

The hair matrix of choice for the analyses is represented by hair, however, when their removal is not possible (for example baldness, zero shaving), alternative collection sites such as the chest, pubis, armpits or the face (beard hair). The collection of samples in private parts of the body requires a careful assessment of the privacy for the subject, while ensuring that the correctness of the collection process is not compromised.

Method for carrying out the sample collection [1]:

The amount of hair needed for the analysis is a few strands of hair, given that the analysis requires a small amount of biological material, since the edx is a survey of very small quantities. The lock of hair to be removed must be fixed with a wire tied as close as possible to the skin, before performing the cut. In the case of short hair this is not possible. In this case, the cut strand is fixed on a sheet of paper with a clip indicating with an arrow the part proximal to the skin.

1. Roll and string a strand of hair with a maximum thickness of a pencil or several thinner strands from the back vertex of the head.
2. Hair should be cut immediately above the skin, as close to the scalp as possible. It is necessary to note the length of the hair strand.
3. The lock of hair must be placed in the appropriate box given by the laboratory. The portion of the lock corresponding to the roots must protrude from the toothed part of the sheet.
4. Each must be ermetically closed in the laboratory given-box.
6. The envelope is accompanied by the collection report and the chain of custody form.

The subjects subjected to sampling were asked not to apply cosmetic treatments such as regular washing with shampoo, the use of products such as conditioner, hair masks, lacquers, foams, jelly, and even dyes, on the skin affected by the sampling itself, permanent, discolorations, since these to varying degrees can alter the concentration of the metals that we are going to look for in the sample.

In addition, the possible use of cosmetic treatments was ascertained at the time of collection and reported in the report of the sample collection.

The sample must be taken in such a way that the proximal part of the sample with respect to the root, in the case of hair, is clearly identifiable.

Hair samples should be stored in a dry environment at room temperature, away from direct sunlight.

Hair samples should not be stored in the refrigerator or freezer, as swelling of the hair matrix may occur.

Hair samples that appear wet at the time of collection must be dried before they are stored in the appropriate bags.

EDX Analysis

Energy dispersive X-ray spectroscopy (EDX) is a technique used for the elemental analysis or chemical characterization of a sample [4]. As a type of spectroscopy, it is based on the investigation of a sample through the interactions between electromagnetic radiation and matter, analyzing the X-rays emitted by matter in response to impact with charged particles. Its characterization capabilities are largely due to the fundamental principle that each element has a unique atomic structure that allows X-rays that are characteristic of an element's atomic structure to be uniquely identified from each other.

The imaging of the surface morphology of the samples can be correlated by an X-ray energy dispersion (EDX) analysis to determine the distribution of the elements present in the periodic table within the sample (5). The Sample, of whatever nature it may be, can be scanned by EDX for a gradient of elements, with incidental X-rays that penetrate the tissue for 3 μ; on the resulting radiation we calculate the Wt% (percentage distribution of the atomic mass of any element) of the sample. The resulting layered mapping represents the distribution of elements on the sample.

Results

The study investigated a total of 15 patients all suffering from various types of Tumor and all belonging to the same geographical area, with particular reference to the Campania region, enclosed in the municipality of Quarto. Biological samples, human hair, collected in the manner described above, from patients in the city of Quarto were analyzed with EDX method. The study was carried out over a period of 6 months, as a preliminary study of a larger study that will include people from the treated municipalities and other people from other municipalities in the same region. The study offered such results:

CODE	*SEX*	*AGE*	*WORK*	*SAMPLING*	*PATHOLOGY*	*CITY*
RAC1	M	67	carpenter	pubis	Pulmonar Tumor	Quarto/Marano
RAC2	M	69	Driver	Scalp	Castelman disease	Quarto
RAC3	M	70	Banking	Scalp	Prostate Cancer	Quarto
RAC4	M	56	Blacksmith	pubis	Kidney tumor	Quarto
RAC5	M	67	Bricklayer	Scalp	Vescical Tumor	Quarto
RAC6	M	65	Bricklayer	Scalp	Pulmonar Tumor	Quarto
RAC7	M	41	Attorney	Scalp	N.D.	Quarto
RAC8	M	73	Policeman	Scalp	Pulmonar Tumor	Quarto
RAC9	M	74	Property developer	Scalp	Metastatic melanoma	Quarto
RAC10	M	78	Driver	Scalp	Colon Tumor/ pulmonary Tumor	Quarto
RAC11	M	61	Landfill overseer	Scalp	Vescical Tumor	Quarto
RAC12	M	54	engineer	Scalp	Linfoma	Quarto
RAC13	M	41	N.D.	Scalp	Pancreas Tumor	Quarto
RAC14	M	73	tiler	Scalp	Colon Tumor	Quarto
RAC15	M	37	N.D.	Scalp	Tumor	Quarto
CTRL NEG	M	40	N.D.	Chest hair	Haelthy	N.D.

Table1: All people examined in that study have an age between 30 and 80 years old, all of the same region, and the same geographical area.

Table of assolute value of Heavy metal in people							
CODE	C	O	Al	Cu	Mo	Tl	Pb
RAC1	49,88	32,39	0,08	0,29	13,26	0	0
RAC2	24,5	32,12	9,28	0,11	7,69	0,53	0,3
RAC3	45,56	28,95	0	0,28	10,89	0	0
RAC4	33,95	28,99	0	0,16	15,64	0	0
RAC5	23,86	22,38	0,28	0,3	13,52	0	0
RAC6	49,56	34,24	0,13	0,17	15,85	0	0
RAC7	25,53	71,33	0,02	0	0,8	0	0
RAC8	24,93	70,59	0,03	0,07	1,2	0	0
RAC9	25,54	71,43	0,14	0	0,54	0	0
RAC10	60,82	14,52	0	0,58	0	19,5	1,9
RAC11	63,42	20,74	0	0,9	0	12,36	1,85
RAC12	62,17	21,19	0	0,49	0	13,66	1,84
RAC13	63,7	19,13	0,11	0,38	0	13,08	1,74
RAC14	60,59	24,19	0	0,25	0	11,9	1,85
RAC15	61,24	20,1	0	0,58	0	14,1	2,2
CTRL NEG	50,33	31,88	0,07	0,07	12,08	0,00	0,00

Table 2: The table shows the values in Nmol / nm; the values that are anomalous both in terms of presence and quantity are indicated in red. The EDX examination was carried out on the intermediate part of the hair by measuring the amount of accumulation of said heavy metals in the last 3-4 months.

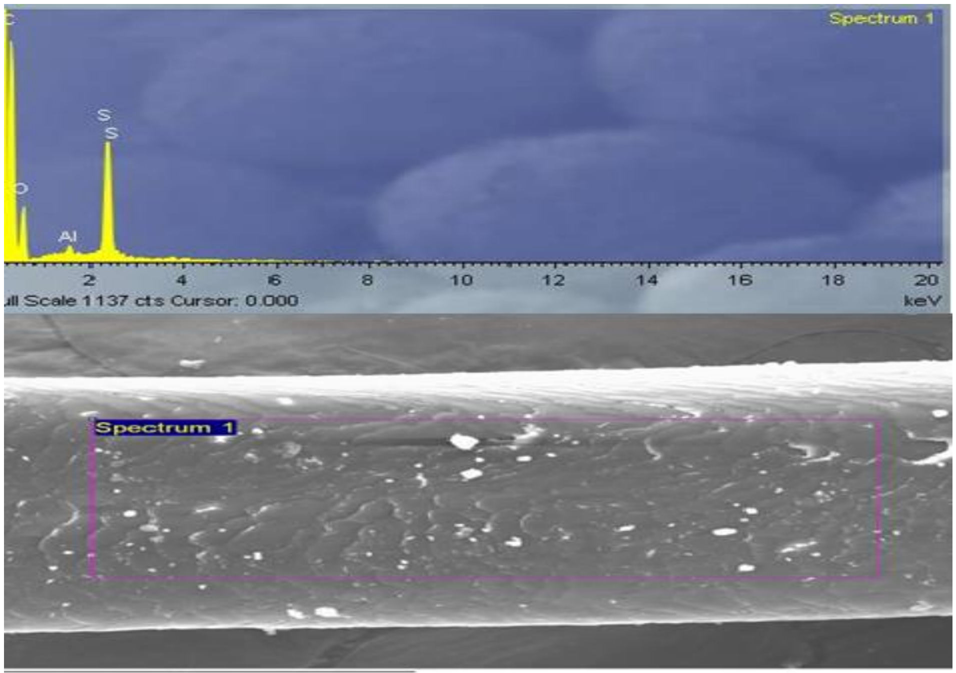

Figure 1: the figure shows an example of the hair area analyzed for the identification of heavy metals with the EDX technique. In association with the spectrum given by the analysis of the selected area

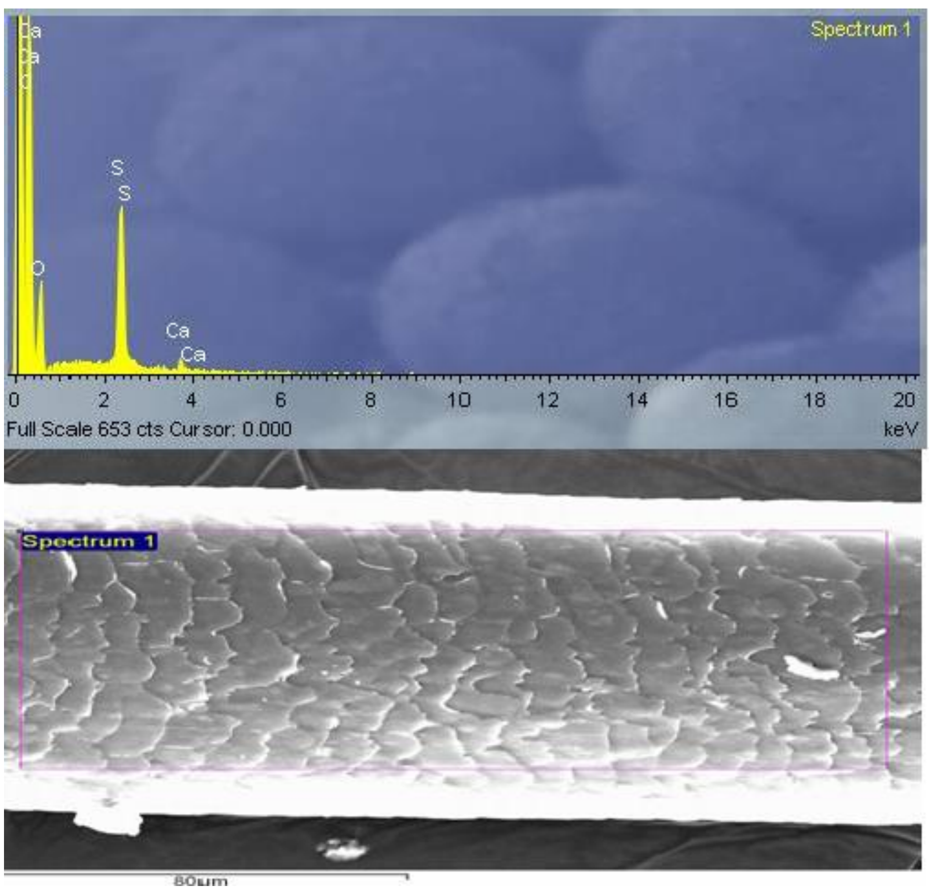

Figure 2: the figure shows another example of the hair area analyzed for the identification of heavy metals with the EDX technique. In association with the spectrum given by the analysis of the selected area

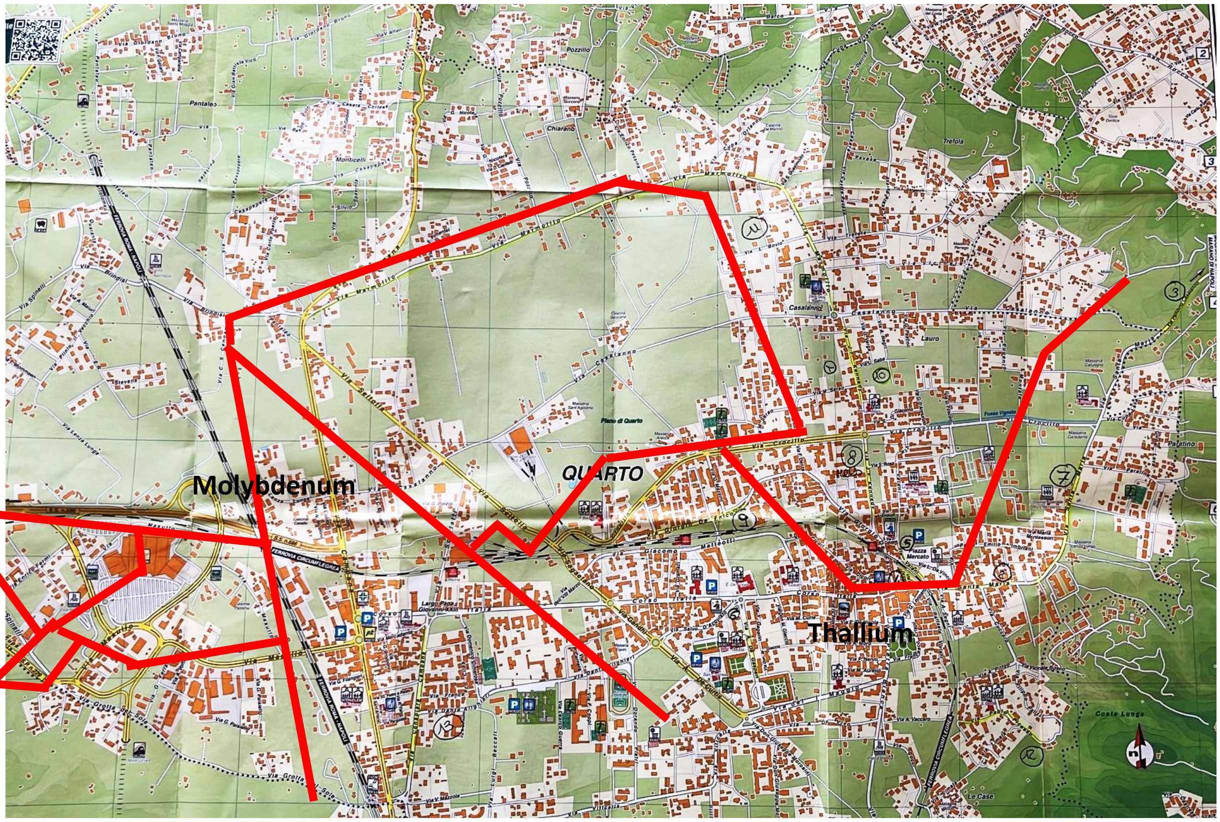

Figure 3: In this map we have signed in red the street where the patient put under exam; as you can see we have delimited two macro-areas one near the railroad (where we observed high level of Molybdenum in hair sample) while in the other macroarea we observed high level of thallium.

Discusion

Thallium (Tl^+) is a ubiquitous natural trace metal considered as the most toxic among heavy metals. The ionic ratio of Tl^+ is similar to that of potassium (K^+), therefore accounting for the replacement of the latter during enzymatic reactions [14]. The principal organelle damaged after Tl^+ exposure is mitochondria. Studies on the mechanisms of Tl^+ include intrinsic pathways altered and changes in antiapoptotic and proapoptotic proteins, cytochrome c, and caspases. Oxidative damage pathways increase after Tl^+ exposure to produce reactive oxygen species (ROS) [13], changes in physical properties of the cell membrane caused by lipid peroxidation, and concomitant activation of antioxidant mechanisms [6]. These processes are likely to account for the neurotoxic effects of the metal. In humans, Tl^+ is absorbed through the skin and mucous membranes and then is widely distributed throughout the body to be accumulated in bones, renal medulla, liver, and the Central Nervous System. Given the growing relevance of Tl^+ intoxication, in recent years there is a notorious increase in the number of reports attending Tl^+ pollution in different countries [15].

In this sense, the neurological symptoms produced by Tl^+ and its neurotoxic effects are gaining attention as they represent a serious health problem all over the world. Through this review, we present an update to general information about Tl^+ toxicity, making emphasis on some recent data about Tl^+ neurotoxicity, as a field requiring attention at the clinical and preclinical levels [7].

Thallium is distributed in bones, kidneys, liver and nervous system, replaces potassium and crosses the blood brain barrier, and can be found in the gonads, has a half-life between 10 and 30 days, the main elimination is via the urine and to an extent minor in milk, nails, hair and feces, chronic toxicity leads to paralysis of the eye muscles, reduced vision, visual disturbances and altered color vision, loss of eyelashes and eyebrows, and then nervous system: depression, anxiety, apathy, insomnia, anorexia, headache, polyneuropathy, tremor, feeling of paralysis, weakness, tingling and muscle aches. In case of chronic inhalation, there is a decrease in Ft4 and thyroid dysfunction.

The ISS (IStituto Superiore di Sanità) indicate the maximum limit of Tl in water is 2microg / L and the use of water contaminated with thallium is 0.04 mg / L. The presence of thallium is associated with sulphides of Pb, barite and Ag.

Heavy metals are particular chemical class of elements and present in the environment. Most of them are harmful even at low concentrations. Several potential mechanisms are implicated in metals toxicity including production of oxygen reactive species (ROS), interaction with thiol groups of proteins, incorrect protein folding and mimicry of the essential elements for intracellular transport, and depletion of antioxidants enzymes [6]. Cadmium is capable to bypass the blood-brain barrier [6]. On the other hand, in human autopsies of individuals with diverse neurodegenerative diseases, significantly higher level of accumulation of Cd in locus ceruleus was observed [7]. Similarly, tin as a heavy metal element, plays a critical role in neurological disease and autoimmune disease [9, 10]. Researchers and in our study, tin was dramatically associated with hearing loss. We assumed that it impaired hearing through the destruction of neurons. Further cellular and animal research is prompted for verification. There are several limitations of our study. Firstly, a cross-sectional study design can only infer the correlation, but causality. Secondly, despite the attempts to adjust hearing-related confounders, unmeasured or unknown covariates may influence the effectiveness of the conclusion. Additionally, the mechanism of the association between hearing loss and trace metals has not been fully addressed. Finally, the data of this study was derived from the United States, the generalizability of the conclusion to other regions or populations should be very cautious. Among the patients analysed can be divided into two groups in particular, in a first group levels above the norm of Molybdenum were found in the analysis, in a second group levels above the norm of Lead and thallium. According to a geographical representation, however, the first group resides near the railway of the municipality of Quarto and the incidence of lung cancer is preponderant in these patients, there could be an environmental correlation from inhalation, the second group resides far from the railway and the incidence of absorption tumors such as urological and haematological ones is preponderant, there could be an environmental correlation from water.

Conclusion

In our Opinion this study is the first step for an deeper and widley investigation about Heavy metal and their effect on people that live in That Region of Italy (Campania) expecially in some areas that are more interested by that phenomena of pollution of the soil [12].

In the next future our investigation could help to identificate the contamination and could allow preventive therapy that can help that population.

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