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The latest trends in hair composition analysis in toxicological studies

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Abstract

Humans have had to deal with toxic substances since times immemorial, and this remains the case in the present day. In case of forensic toxicology, of interest are those substances whose possession, use or effects of either imply an offense, a crime or a threat to life. Biological materials used in toxicological analyses are usually blood, urine and the vitreous body of the eye. In addition to the above-mentioned classic materials used for tests aimed at determining the presence of a given xenobiotic, alternative materials, such as hair, are increasingly being used.

The primary goal of forensic and clinical toxicology is to look for a causal relationship between the presence of a toxic agent in the system and the specific biological effect caused by it. Carrying out the above objective requires the toxicology analyst to choose the appropriate diagnostic material and the appropriate analytical methods. Toxicological analysis can be performed for any type of material, including hair, which is an excellent sample for determination of individual substances.
One of the most important tasks in the field of forensic toxicology is the analysis of addictive substances, whose presence or absence in the human body is one of the elements of a medico-legal report.

Keywords: hair; toxicology; analysis; forensics; alternative material

Introduction

The first elemental analysis of hair was carried out by Hoppe in 1858 [1], who marked arsenic (As) in hair of a body exhumed after 11 years. This event gave rise to the hypothesis that the content of trace elements in hair may reflect their presence in the human body. However, in the following decades, this material was not selected for testing; instead, the focus was on blood and urine analysis. It was not until the second half of the 20th century, when Goldblum marked amphetamine in guinea pig hair and, later, Baumgartner successfully extracted opiates from hair structure, that hair began to be considered a useful test material, not only for determination of trace elements, but also other xenobiotics, including drugs and narcotics. [1] With the development of new analytical techniques, new perspectives related to hair analysis appeared. For example, in 1980, Klug verified radioimmunological results using chromatography for codeine and morphine in the range of 0.1-10 ng / mg. [2] Nowadays, hair analysis is well established as a test with a wide range of applications in toxicological, nutritional, clinical, archaeological and forensic research. [3,4] The aim of this work is to present the current knowledge on applications of toxicological hair analysis and the argumentation justifying this type of analysis.

Hair as an alternative test material

The use of alternative biological materials in the field of toxicology was first described in 1979, using hair analysis to document chronic drug use. From then on, the use of alternative biological

materials for toxicological analysis has gained enormous significance in clinical and forensic toxicology. Alternative samples present a number of advantages over traditional materials used in forensic toxicology; for instance, collection of such materials is a non-invasive and painless process, adulteration of samples is difficult, and they have a wider range of detection (so-called "detection window"). [2,3,5]

Of the various alternative materials used in toxicological assessment, hair is the most important. It is one of the most frequently studied biological traces. [1,3,6]

Its unique properties, such as durability and non-invasive nature of collection, allow the analysis to "reproduce" the history of taken xenobiotics – hair is a unique material in retrospective tests in cases of chronic exposure to xenobiotics, and is characterized by a wider detection window compared to blood or urine. [1-4,7]

Due to simplicity of sampling in a non-invasive manner, hair analysis is widely used in drug tests and drug rehabilitation programs, as well as analysis of trace elements.[8] Due to lack of hair metabolism and the possibility of conducting long-term exposure analysis through segmental analysis, hair is also a good matrix for post-mortem examinations in forensic medicine. A unique feature of this matrix is the ability to accumulate xenobiotics with which there was contact in the past, which provides examiners with a longer time window (months or even years). Due to the fact that hair growth is similar in every human and is approx. 1.06 cm per month [9], interpretation of results is simplified. Analysis is easiest when a xenobiotic is detected only in the relevant segment corresponding to the exposure time, and absent in all others. [10] Because hair is a dynamic tissue structure and its development includes three phases: the anagen (growth) phase - the longest phase, lasting 2-7 years and encompassing about 90% of hair, the catagen (transitional) phase, lasting 14-21 days, and the telogen (resting) phase, lasting up to 90 days [11], certain xenobiotics can be detected in neighboring segments. [9] This phenomenon is affected not only by the uneven growth rate, but also the secretion of sweat and sebum and improper hair collection for the test.

In addition, hair analysis provides a non-invasive way to easily monitor sampling and ensures reduced risk of adulteration, easy storage and transportation and lower risk of exposure to biological hazards. In addition, hair analysis is easier than previously used matrices, because the analyte is usually present in higher concentrations in the hair than in blood and urine. [8] The concentration of elements in the serum often does not correspond to their content in the whole body, because their concentrations in the blood are controlled by mechanisms which maintain homeostasis. During formation of hair fibers, elements are permanently incorporated into the keratin structure and consequently are excluded from metabolic processes. [4] In

addition, it is possible to use hair as a material in situations when classic matrices are not available, for example in case of decomposing bodies. Provided that hair is not cut, it is easy to obtain a second sample if results are inconclusive. [5] The usefulness and reliability of hair analysis depends on the ability to identify and quantify xenobiotics and metabolites present in hair due to direct contact with the substance, not due to passive exposure. When interpreting the results, one should take into account the possible application of cosmetics or beauty treatments, such as dyeing or bleaching, which cause degradation of substances. However, it is difficult to determine the exact amounts of compounds due to natural variability of hair composition depending on age, sex, hair color, ethnic and geographical origin, nutritional factors, etc. For this purpose, certified reference materials (CRM) are used; for instance, there are certified concentration values for 55 elements as well as methyl mercury in hair, or certified concentration values for determination of analyte recovery. [8] However, researchers suggest that, in addition to age and gender, as separate groups, additional analyses for each country or region should be carried out when setting reference values. [12] The possibility of external hair contamination should also be considered, e.g. passive smoking or polluted environment. Uncertainties regarding the mechanisms of penetration of trace elements into hair and lack of correlation between their concentration in internal organs and their concentration in hair are still grounds for "discrediting" the results obtained from this matrix. In addition, lack of cohesive standards and guidelines for preparation of samples is a limitation in the analytical procedure. Nevertheless, the development of quantitative research in this area over the last 10 years, with a particular emphasis on the use of inductively coupled plasma mass spectrometry (ICP-MS), opens the way for application of this matrix in multiple tests. [1,8,13,14]

Hair structure

Hair structure can be analyzed by taking into account either spherical or horizontal cross-section. In order to evaluate the time elapsed since contact with xenobiotics, knowing the horizontal structure of hair seems more important. Hair consists of two main parts: the shaft – a stem, which protrudes above the surface of the skin, and the root, located within the skin. The hair root resides in a sheath-like epidermal cavity called the hair follicle, which ends with a thickening called the hair bulb. This is where hair and its epithelial sheaths form from the matrix. A vascularized hair papilla, containing numerous blood vessels, protrudes from the base of the bulb. Depending on the phase of life, hair can be very developed, e.g. during the anagen phase, or may be slowly disappearing, which occurs during the catagen phase. The cells just above the top of the papilla produce hard keratin. Newly formed cells push the older ones up.

This way, dead cells filled with keratin protrude from the skin surface and form the hair shaft. The arrector pili muscle is attached to the hair follicle in a line running towards the skin surface; the sebaceous gland opening is located above it. Above the gland opening, the follicle is called the infundibulum. Here, hair is loosely set in the skin. [4,11]

In order to analyze the penetration depth of the substance and its affinity for hair, the structure of the cross-section and the chemical composition of individual layers should be considered. Hair is made of 65-95% protein (mainly keratin), 1-9% fats and 0.1-5% pigments – melanin; remaining components are trace elements, polysaccharides and water (15-35%). Cross-section of hair has a layered structure. Going from the outside, this structure consists of the hair follicle, followed by the root sheath (outer and inner), and finally, the hair shaft in the center. The hair shaft consists of the cuticle, the cortex and the hydrophobic medulla. [4,11]

The cuticle is the outer part of the hair, built of single, squamous, keratinized cells with overlapping scales, which provide protection against moisture loss and environmental factors. These cells do not contain nuclei or pigment, and sebum accumulates between them. Fats, which are components of the hair cell membrane, may bind nonspecifically to certain compounds, e.g. cocaine. The cuticle may also contain small gas bubbles. The cortex is the main component of hair. It is built of elongated, keratinized akaryocytes. Nuclei can only be found within the root. The main building component – keratin – is made of amino acids, which means that it has a greater affinity for alkaline compounds, e.g. cocaine, nicotine and morphine, and smaller for compounds of acidic nature, e.g. Δ9-tetrahydrocannabinol (THC) or acetylsalicylic acid. Most metals have a high affinity for sulfhydryl groups of amino acids, which constitute keratin, and are therefore easily incorporated into hair. [6] Pigment cells – melanocytes – are located between the cells. They produce melanin, which, as hair grows, passes from the bulb to the shaft. It is suspected that melanin is one of the binding sites for xenobiotics in hair, and the interaction takes place via van der Waals attraction between the aromatic rings of melanin and the compound being incorporated. Melanin, due to the fact that it is a polymer with negatively charged carboxyl groups, can bind via ionic bonds at physiologic pH. [8]

The hair medulla lies in the axis of hair and is often absent in human hair. Therefore, its role in the process of identifying xenobiotics is negligible. The medulla consists of 1-2 rows of oval cells, with air bubbles in between. Unlike the hair shaft, the root also has additional concentric layers, called sheaths. They are divided into the outer root sheath, which surrounds the hair and the inner root sheath, ends just above the hair papilla and is connected to the sebaceous gland

and the arrector pili muscle, and the inner root sheath, which is divided into several layers and ends at the sebaceous gland opening. Remnants of sheath decomposition along with secretion of the sebaceous gland form the sebum. [4,8,15]

There are several potential mechanisms for penetration of substances into hair. The most important ones include: passive diffusion from capillaries during hair formation and penetration via diffusion through sebum or sweat secretion to hair follicles after exposure from external environment. [16] According to the passive diffusion model, the elimination of drugs present in hair should be delayed for a few days, because this is how long it takes for a new hair to break through to the skin surface. [7]

The process of diffusion into hair is influenced by both chemical properties of the xenobiotic and by individual physiological features. The most important of these are: hair melanin content and lipophilicity and alkalinity of the substance itself. Hair with a higher melanin content will contain higher amounts of xenobiotics, and lipophilic and alkaline particles will more easily penetrate into the hair structure. During analysis, it should be taken into account that concentration of compounds in hair also depends on: gender, hair color, eating habits, age and lifestyle. [17]

With the above in mind, the Society of Hair Testing (SoHT), founded in 1995, develops standards aiming to define, update and harmonize the rules and procedures related to hair analysis. In Europe, two more such programs are also available: a) HAIRVEQ (an external quality control scheme for drugs of abuse in hair) set up by the Istituto Superiore di Sanità in Rome, Italy, in cooperation with the Institut Municipal d'Investigaciò Mèdica from Barcelona, Spain, and b) GTFCh (Gesellschaft für Toxikologische und Forensische Chemie) created by the German Society of Toxicological and Forensic Chemistry. [18]

Collection of material for testing and determination

Biological material, such as hair, requires proper preparation [Fig 1].

Before the actual analysis stage, it is recommended to wash the hair. [7,18] Hair testing consists of several stages: decontamination of sample from external impurities, drying, hair homogenization, extraction of determined substances from hair matrix. It is assumed that hair grows 1 cm ± 0.2 cm / month, so using it as a diagnostic material is justified, which allows making observations for dates going back up to ten, or sometimes up to twenty, months, usually between 1 and 6 months. Unfortunately, hair is not suitable for assessment of acute poisoning. [7,18] There are recommendations for hair sampling for toxicological analysis [7,18]

An important step in the analysis of the sample is the correct collection of material for testing. It is necessary to collect as much hair in the anagen phase as possible, because the papilla is heavily vascularized in this phase. In case of human hair, samples are taken from the back of the head, because it is the region with the smallest variability in growth rate. The material should be taken as close to the scalp as possible, using scissors made of polyethylene, titanium or tantalum. The collected hair are tied with a thread or an elastic band, so as to mark the bottom end, which was cut off near the skin. In cases where head hair is not available, hair can be collected from other parts of the body; however, changes in growth phases should be taken into account. Considering that hair is a stable diagnostic material, hair samples can be stored in a plastic container, an envelope or aluminum foil, shielded from light, at room temperature. [7,8,18]

Fig. 1. Procedure for collecting hair for toxicological analysis. 1A – Separate a pencil-wide strand of hair, from the back of the head, tie with a string (about 1 cm from the scalp), cut off with sterile scissors; 1B – place the sample on aluminum foil so that the end protrudes beyond the foil; 1C – wrap the foil in the shape of an envelope, do not wrap the sample too tightly; 1D – Complete the appropriate sample collection form and place all items in a separate paper envelope. (photo: M. Szopa)

Before testing, samples should be decontaminated using detergents or organic solvents to remove lipophilic impurities, which should be followed by decontamination using aqueous solvents or methanol. However, the use of methanol should be minimized, because it can extract the analytes and thus cause their loss. The number of cleaning stages depends on the degree of contamination. Washings should be stored at 4° C for later analysis. After washing, the hair are dried and then powdered in a ball mill (Fig.2) or cut into small pieces. There are two options for extraction: the whole sample or selective isolation of a selected component. Techniques such as filtration, Soxhlet apparatus extraction, SPE (solid-phase extraction) or SFE (supercritical fluid extraction) can be used for this purpose. [3,4,6,7,18]

Fig. 2. Laboratory ball mill used for hair processing in toxicological tests (photo: M. Szopa)

The purification and derivatization process is unnecessary, although it has been the object of interest lately due to the possibility of obtaining of more satisfying results. Derivatization is a process aimed at modifying chemical compounds, through which a new compound is formed, which is easier to mark using analytical methods. Sample testing should be carried out using analytical methods allowing accurate and precise results.

Continued innovation and improvement of analytical tools allows the use of increasingly complex procedures with smaller hair samples. In particular, the constant development of new chromatographic technologies and mass spectrometry has significantly improved the application of hair analysis in the field of clinical and forensic toxicology. The emergence of ultra-high performance liquid chromatography (UHPLC), along with TOF and Orbitrap mass analyzers, is a technological milestone towards progressive determination of analytes in hair matrix. High sensitivity and the ability to analyze a complex mixture in mass spectra allows analysis of ever smaller amounts of substances. Recently, the LC-MS-MS (liquid chromatography-tandem mass spectrometry) method was developed, with the aim of

identifying as many as 87 psychoactive drugs and metabolites in 20 mg of hair, reaching the limit of quantification (LOQ) of 0.3-45 pg / mg; the method was used for real post-mortem specimens. [1,5,8,14,19] A considerable achievement is the ability to detect xenobiotics at a particularly low concentration, e.g. THC, whose concentration in the hair is often below 1 μg / mg, and the values for proving cannabis abuse are set at 50-100 μg / mg. While traditional methods for determining THC in hair are based on GC-ECNI-MS-MS (Gas chromatography in combination with electron capture negative ion mass spectrometers), following chemical derivatization new, alternative approaches have been proposed in order to achieve an extremely low level of detection limits. These include GCxGC-MS (Shimadzu's Comprehensive GC-MS) and LC-MS-MS. Despite the many achievements related to this issue, analysts continue to face multiple challenges. [1,15,20]

Due to the broad development of analytical techniques for hair testing, there is an increasing interest in using this matrix in many new fields. The following are examples of applications of toxicological hair analysis. [10,14,20–31]

Tab. 1. Selected substances tested for in hair

Application	Subject of tests	Method
Forensic medicine	Flunitrazepam	GC-MS
Anthropology	Cocaine, Morphine	GC-MS
Anti-doping tests	Anabolic androgenic steroids, β2-agonists, hormonal antagonists, glucocorticoids	LC-MS-MS
Testing of employers, drivers	Tetrahydrocannabionols, amphetamine, methamphetamine, cannabinoids, cocaine, opiates, methadone and benzodiazepines	GC-MS LC-MS-MS
Monitoring of addiction therapies	6-acetylmorphine (6-MAM), amphetamine, benzoylecgonine (BZE), cocaine, codeine, dihydrocodeine, EDDP (methadone metabolite), methadone and morphine	GC-MS
Epidemiology and environmental exposure	Exposure of workers to hydroxyl derivatives of polycyclic aromatic hydrocarbons (OH-PAH)	GC-MS
Agriculture, industry	Pesticides	HRGC-HRMS, GC-ECD i GC-MS,
Medicine	Changes in the amounts of trace elements (Co, Cd, Zn, Fe, Cu, I), e.g. in autism, cancers, hypertension, diabetes, kidney diseases	ICP-MS, ASA, NAA

GC-MS – Gas Chromatography-Mass Spectrometry; **HS-SPME-GC-MS-MS** – Headspace Solid Phase Microextraction with Gas Chromatography-Tandem Mass Spectrometry; **ICP-MS-CRC** – Inductively Coupled Plasma Mass Spectrometry-Collision/Reaction Cell; **HRGC-HRMS** – High Resolution Gas Chromatography-High Resolution Mass Spectrometry; **AAS** – Atomic Absorption Spectrometry; **NAA** – Neutron Activation Analysis

Summary

Toxicological assessment aiming at identification and quantitative analysis of xenobiotics primarily uses body fluids (blood and urine – for pre-mortem tests) as well as internal organ specimens (for post-mortem tests) as biological material. These materials have been termed "classic". Observation of the directions of development of toxicology indicates interest in other types of biological material, such as saliva, hair, nails, vitreous body or meconium, which are referred to as alternative materials and are now being researched in terms of usefulness for toxicological assessment in comparison with classic materials. Toxicological analysis of alternative materials broadens the scope of assessment, giving it a comprehensive character, and thus provides additional options for giving expert opinions in the field of forensic toxicology.

The development of analytical methods in hair analysis and the widespread interest in using this biological material displayed by scientists over the last 10 years contributes to progress in the field of toxicology. Creation of guidelines for individual stages of hair testing makes this method very reliable and increasingly used in various fields, including criminology, anthropology and medicine.

References

[1] Moeller MR. Drug detection in hair by chromatographic procedures. J Chromatogr B Biomed Sci Appl 1992;580:125–34. doi:10.1016/0378-4347(92)80532-U.

[2] Sachs H. History of hair analysis. Forensic Sci Int 1997;84:7–16. doi:10.1016/S0379-0738(96)02043-9.

[3] Kintz P, Salomone A, Vincenti M. Hair analysis in clinical and forensic toxicology. 1st ed. 2015.

[4] Wołowiec P, Michalak I, Chojnacka K, Mikulewicz M. Hair analysis in health assessment. Clin Chim Acta 2013;419:139–71. doi:10.1016/j.cca.2013.02.001.

[5] Łuczak-Zielkiewicz I, Szutowski MM. BIULETYN Wydziału Farmaceutycznego Warszawskiego Uniwersytetu Medycznego WARTOŚĆ DIAGNOSTYCZNA WŁOSÓW. Biul Wydz Farm WUM 2013;8:56–64.

[6] Popescu C, Höcker H. Hair—the most sophisticated biological composite material. Chem Soc Rev 2007;36:1282. doi:10.1039/b604537p.

[7] Society of Hair Testing. Recommendations for Hair Testing in Forensic Cases Society of Hair Testing. Forensic Sci Int 2004;145:83–4.

[8] Pozebon D, Scheffler GL, Dressler VL. Elemental hair analysis: A review of procedures and applications. Anal Chim Acta 2017;992:1–23. doi:10.1016/j.aca.2017.09.017.

[9] LeBeau MA, Montgomery MA, Brewer JD. The role of variations in growth rate and sample collection on interpreting results of segmental analyses of hair. Forensic Sci Int 2011;210:110–6. doi:10.1016/j.forsciint.2011.02.015.

[10] Wang X, Johansen SS, Nielsen MKK, Linnet K. Hair analysis in toxicological investigation of drug-facilitated crimes in Denmark over a 8-year period. Forensic Sci Int 2018;285:e1–12. doi:10.1016/j.forsciint.2018.01.021.

[11] Tobin DJ. Biology of Hair. The Biogenesis and Growth of Human Hair. In: Tobin DJ, editor. Hair Toxicol., Cambridge: Royal Society of Chemistry; 2005, p. 1–33. doi:10.1039/9781847552518-00001.

[12] Peña-Fernández A, González-Muñoz MJ, Lobo-Bedmar MC. Evaluating the effect of age and area of residence in the metal and metalloid contents in human hair and urban topsoils. Environ Sci Pollut Res 2016;23:21299–312. doi:10.1007/s11356-016-7352-3.

[13] Ahrens BD, Starcevic B, Butch AW. Detection of Prohibited Substances by Liquid Chromatography Tandem Mass Spectrometry for Sports Doping Control. Methods Mol. Biol., vol. 902, 2012, p. 115–28. doi:10.1007/978-1-61779-934-1_10.

[14] Fisichella M, Morini L, Sempio C, Groppi A. Validation of a multi-analyte LC–MS/MS

method for screening and quantification of 87 psychoactive drugs and their metabolites in hair. Anal Bioanal Chem 2014;406:3497–506. doi:10.1007/s00216-014-7763-2.

[15] Samanidou VF. The challenges in hair analysis from the perspective of an analytical chemist. J Appl Bioanal 2016;2:103–7. doi:10.17145/jab.16.014.

[16] Reicher M (1888-1973), Bilikiewicz T, Hiller S, Stołyhwo E, Sieńkowski E, Łasiński W, et al. Anatomia ogólna : kości, stawy i więzadła, mięśnie. Wydawnictwo Lekarskie PZWL; 2010.

[17] Mikulewicz M, Chojnacka K, Gedrange T, Górecki H. Reference values of elements in human hair: A systematic review. Environ Toxicol Pharmacol 2013;36:1077–86. doi:10.1016/j.etap.2013.09.012.

[18] Federal Register : Agencies - Substance Abuse and Mental Health Services Administration n.d. https://www.federalregister.gov/documents/2018/07/19/2018-15450/substance-abuse-and-mental-health-services-administration (accessed August 24, 2018).

[19] Workplace E, Testing D. European Guidelines for Workplace Drug and Alcohol Testing in These guidelines for Legally Defensible Workplace Drug Testing have been prepared European Guidelines for Workplace Drug and Alcohol Testing in Hair 2015:1–23.

[20] Moeller MR, Fey P, Wennig R. Simultaneous determination of drugs of abuse (opiates, cocaine and amphetamine) in human hair by GC/MS and its application to a methadone treatment program. Forensic Sci Int 1993;63:185–206.

[21] Afridi HI, Kazi TG, Kazi N, Kandhro GA, Baig JA, Shah AQ, et al. Evaluation of Toxic Elements in Scalp Hair Samples of Myocardial Infarction Patients at Different Stages as Related to Controls. Biol Trace Elem Res 2010;134:1–12. doi:10.1007/s12011-009-8450-6.

[22] Kazi TG, Jalbani N, Kazi N, Arain MB, Jamali MK, Afridi HI, et al. Estimation of Toxic Metals in Scalp Hair Samples of Chronic Kidney Patients. Biol Trace Elem Res 2009;127:16–27. doi:10.1007/s12011-008-8222-8.

[23] Yasuda H, Tsutsui T. Assessment of infantile mineral imbalances in autism spectrum disorders (ASDs). Int J Environ Res Public Health 2013;10:6027–43. doi:10.3390/ijerph10116027.

[24] Man CK, Zheng YH. Analysis of trace elements in scalp hair of mentally retarded children. J Radioanal Nucl Chem 2002;253:375–7. doi:10.1023/A:1020405100202.

[25] Benderli Cihan Y, Sözen S, Öztürk Yıldırım S. Trace Elements and Heavy Metals in Hair of Stage III Breast Cancer Patients. Biol Trace Elem Res 2011;144:360–79. doi:10.1007/s12011-011-9104-z.

[26] Thevis M, Geyer H, Tretzel L, Schänzer W. Sports drug testing using complementary matrices: Advantages and limitations. J Pharm Biomed Anal 2016;130:220–30. doi:10.1016/j.jpba.2016.03.055.

[27] Appenzeller BMR, Mathon C, Schummer C, Alkerwi A, Lair M-L. Simultaneous determination of nicotine and PAH metabolites in human hair specimen: A potential methodology to assess tobacco smoke contribution in PAH exposure. Toxicol Lett 2012;210:211–9. doi:10.1016/j.toxlet.2011.11.022.

[28] Tan C, Chen H. Screening of Prostate Cancer by Analyzing Trace Elements in Hair and Chemometrics. Biol Trace Elem Res 2011;144:97–108. doi:10.1007/s12011-011-9038-5.

[29] Khalique A, Shah MH, Jaffar M, Shaheen N, Tariq SR, Manzoor S. Multivariate Analysis of the Selected Metals in the Hair of Cerebral Palsy Patients Versus Controls. Biol Trace Elem Res 2006;111:11–22. doi:10.1385/BTER:111:1:11.

[30] Joo N-S, Kim S-M, Jung Y-S, Kim K-M. Hair Iron and Other Minerals' Level in Breast Cancer Patients. Biol Trace Elem Res 2009;129:28–35. doi:10.1007/s12011-008-8281-x.

[31] Afridi HI, Kazi TG, Kazi NG, Jamali MK, Arain MB, Sirajuddin, et al. Evaluation of cadmium, lead, nickel and zinc status in biological samples of smokers and nonsmokers hypertensive patients. J Hum Hypertens 2010;24:34–43. doi:10.1038/jhh.2009.39.