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Evaluation of essential and toxic metals by ultrasound-assisted acid leaching from scalp hair samples of children with macular degeneration patients

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Abstract

Background: The causes of night blindness in children are multifactorial and particular consideration has been given to childhood nutritional deficiency, which is the most common problem found in underdeveloped countries. Such deficiency can result in physiological and pathological processes that in turn influence hair composition.
Method: An ultrasonic-assisted acid leaching procedure was developed as a sample pretreatment for the determination of Zn, Cu, Cd, As and Pb in human scalp hair samples of night blindness male children with age between 5 to 15 y and compared with the children without vision anomalies that lived in the same localities. The effects of different factors on acid leaching of metals, such as preintensification time (without ultrasonic stirring) after treatment of acid mixture, exposure time to ultrasound and temperature of the ultrasonic bath have been investigated. The proposed method was validated by certified reference samples of scalp hair CRM 397. The wet acid digestion method was used to obtain the total metal concentration in both scalp hair and CRM samples. Cu and Zn in leachates and digests were measured by flame atomic absorption spectrometry (FAAS) using a conventional air/acetylene flame, while Cd and Pb were determined by electrothermal atomic absorption spectrometry (ETAAS) under optimized conditions.
Results: It was observed that at optimal conditions, the recovery for Zn, Cd, Pb, As and Cu were 98%, 98.5%, 96%, 97.2% and 94% respectively. The mean values of Zn and Cu in scalp hair samples of children having night blindness were significantly lower as compared to normal healthy children (p for Zn $<$ 0.001 and Cu $<$ 0.003), while the level of toxic metals As, Cd and Pb were significantly higher in children having ocular problems as related to normal children (p As $<$ 0.0074, Cd $<$ 0.001 and lead $<$ 0.004).
Conclusion: These data present guidance to clinicians and other professional investigating deficiency of essential trace metals and excessive level of toxic metals in biological samples.

Keywords: Trace and toxic elements; Night blindness; Children; Age groups (5 – 15); Atomic absorption spectrophotometer

1. Introduction

With the recent development of analytical techniques of great power and sensitivity, the significance of the levels of trace elements in human hair has attracted the attention of many disciplines including the environmental sciences [1]. The interest in human hair as a biological material for clinical studies and diagnostics has increased in recent years due to certain advantages offered over other specimens such as blood or urine [2–4].

Hair analysis is a simple diagnostic technique, based on the idea that hair provides vital clues about nutritional imbalances elsewhere in the body. It is also used to detect environmental toxins before overt symptoms appear because metal concentrations in hair are usually tenfolds higher than

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in other biological materials [5–8]. Most enzymes in human body are proteins, along with an essential mineral and possibly a vitamin. If essential minerals are lacking, the corresponding enzymes can not function properly [9–12].

Vitamin A has been reported to be necessary for normal growth and repair of body tissues, protect the mucous membranes of the mouth, nose, throat and lungs, counteracts night blindness and weak eyesight [13–15]. Zinc is necessary for the enzyme that activates vitamin A in the vision. Without zinc, vitamin A can not be converted to its active form [16] and it is required in hepatic synthesis and secretion of the retinol binding protein (RBP), a primary plasma transport protein for retinal (vitamin A). Hence, Zn deficiency may be related with the etiology of night blindness in childhood [17–19].

Determination of trace metals in biological samples requires the use of sensitive and selective techniques such as electrothermal atomic absorption spectrometer (ETAAS), using sample preparation strategies addressed to shortening and simplifying the stages previous to analysis. The determination of metals by AAS involves acid digestion of the biological samples, with effectively removing the organic matter [20].

However, scalp hair analysis in the diagnostic of such ocular problem is not a routine methodology. This can be a result from the lack of reference values for the concentrations of trace elements in human hair. External contamination of hair samples by dust and sweat and cosmetic treatments, are other drawbacks that have to be taken into account when metal determination in human hair is performed. These limitations surpassed using standard procedures accepted by the international community for both hair sampling and hair washing processes [21]. Different sample pre-treatments for the analysis of trace elements in human hair samples, such as acid digestion procedures, have also been developed [22].

The extraction procedure of elements by the action of an acid and an oxidant agent, named as acid leaching, appears to be an interesting sample pre-treatment method. Leaching is not a total decomposition and leachable recoveries of analytes are generally lower than total concentrations. Recoveries can only achieve total values, if the elements are completely soluble in the leaching solvent [23]. The extraction of metals in solid biological samples with acid and oxidant mixture can enhance with the use of ultrasound radiation [24]. The main advantage of ultrasonic assisted leaching of metals in hair samples is the requirement of lower acid concentrations, when compared with wet acid digestion procedures to achieve extraction of metals. Thus, the consumption of mineral acids and nitrous oxide vapors produced during wet acid digestion on a hot plate, are reduced. In addition, the time required for sample preparation is also diminished [25].

As many variables are involved throughout the acid leaching process, such as, preintensification time (time intervals for the treatment of hair samples with the acid mixture at room temperature without ultrasonic stirring), duration of sonication and temperature of ultrasonic bath, the optimization of each variable was studied. Metals determination in leachate was carried out by FAAS/ETAAS, the results were compared with those obtained by conventional wet acid digestion.

We validated a new and less time expensive method to analyze trace and toxic metals in large number of biological samples (scalp hair) and also to evaluate the deficiency or excessive amount of metals, related to different physiological disorders such as night blindness in children. The differences among the concentration of essential trace and toxic metals in scalp hair samples of normal children and patients with night blindness were investigated.

2. Materials and methods

2.1. Reagents and glassware

Ultrapure water obtained form a Milli-Q purifier system (Millipore Corp., Bedford, MA) was used throughout the work. Nitric acid and hydrogen peroxide were analytical reagent-grade from E. Merck® and were checked for possible trace metal contamination. Standard solutions of zinc, copper, lead, arsenic and cadmium were prepared by dilution of certified standard solutions (1000 ppm, Fluka Kamica®) of corresponding metal ions. The standard solutions were prepared with 2 N nitric acid. All glassware and plastic material used was previously treated for a week in supra pure nitric acid and rinsed with double distilled water and then with ultra-pure water.

2.2. Apparatus

The ultrasonic extractions were carried out with a Sonicor, Model No. SC-121TH, programmable for temperature ranging from 0 to 90 °C with intensification frequency of 35 kHz. A total volume of 4 l, was used to induce the acid leaching process. Centrifugation was carried out to separate the supernatant from the sample extracts with a WIROWKA Laboratoryjna type WE-1, nr-6933 centrifuge; speeds range 0–6000 rpm. Metals were determined in digests and leachate using either a FAAS or ETAAS of Hitachi Ltd., Model 180-50, S.N.5721-2, with a deuterium lamp background corrector, linear (least square) mode and equipped with a graphite furnace GA-03. A Hitachi Model 056 recorder was used to store analytical data of the metals under investigation. Hollow cathode lamps (Hitachi) operating at recommended current were used for all the metals.

2.3. Sampling

The study was conducted on 110 children (5–15 y), male, registered in the hospital as patients with ocular