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Toxicology

Essential metals profile of the hair and nails of patients with laryngeal cancer

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ABSTRACT

Trace elements have an impact on numerous physiological processes. The monitoring of their levels in the organism allows you to detect not only their deficiencies, but also several illnesses. The aim of this study was to compare the levels of essential elements (calcium, magnesium, zinc, copper, iron, manganese) in hair, nails and serum of both patients with laryngeal cancer and healthy people. The determination of six metals was performed by an inductively coupled plasma mass spectrometry (ICP-MS) and an inductively coupled plasma optical emission spectrometry (ICP-OES). The concentration of essential elements in hair and nails of the control group was statistically significantly higher than in the group of patients with laryngeal cancer. In the case of serum, differences were found between the patients and controls in respect of the level of three metals. The results of principal component analysis (PCA) revealed the strong and similar clustering behavior of essential elements in hair and nails. The metals did not correlate between two alternative materials. The present study indicated that, using the level of essential elements in hair and nails as a basis, it is possible to distinguish cancer patients from healthy people. The alternative materials are independent of homeostasis and therefore seem to be more useful in the detection of diseases and mineral deficiencies in human than the classical biological materials, such as blood.

Introduction

Between 2006 and 2010, there has been a slight decrease in the incidence of malignant cancer in men and no change in women [1]. However, cancer was still the second leading cause of death, after heart diseases, in 2010 in the U.S. [2]. According to prognosis, by 2030 over 26 million new cancer cases will be diagnosed worldwide, and approximately 17 million people will die from this disease [3]. Among head and neck carcinomas, which were the eighth leading cause of cancer death, the most common is laryngeal cancer [4]. Recently, a tendency toward greater prevalence of laryngeal cancer (incidence) has been observed due to the increase in oropharyngeal cancer attributed to human papilloma virus (HPV) infection [5,6]. The incidence of laryngeal cancer is influenced by both exogenous and endogenous factors, including genetic ones. A very important role in carcinogenesis is played by metals. Toxic metals have a negative effect on cellular components and enzymes involved in their metabolism, detoxification and repair processes, while the contribution of essential elements can be interpreted in terms of mineral deficiency and the problems associated with it. Appropriate concentrations and proportions of micro- and macronutrients are undoubtedly the most important factors that affect homeostasis. This dynamic equilibrium state is crucial for maintaining the proper functioning of the human body [7]. Essential elements are described as metabolic and functional food ingredients required to sustain life. Both shortage and excess

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of trace elements lead to physiological disorders and a high concentration of metals may exert toxic effects [8]. Ions of zinc (Zn), cooper (Cu) and magnesium (Mg) are components of antioxidant enzymes, acting as a natural oxidant barrier, a lack of which (elements) weakens it, and the resultant oxidative stress promotes tumor formation.

The mentioned elements play a special role in maintaining the integrity of cell membranes, and in cellular respiration [9]. Zinc is a component of superoxide dismutase (SOD), which is a key enzyme for protection against oxidative stress. Copper has similar antioxidative functions to Zn and is found in enzymes involved in redox processes [10]. Magnesium is involved in the metabolic pathways of carbohydrates, nucleic acids, proteins, and lipids. This element also stabilizes the structure of DNA and affects the transcription of RNA. Calcium (Ca) plays an important role in biological tissues and fluids as a part of the intracellular and extracellular fluid and various membrane structures [11].

Therefore, the biological monitoring of essential metals in the human population has become an important issue. In the case of cancer, the assessment of concentration of metals is justified and necessary.

Unfortunately, analysis of the blood does not reflect the actual concentration in the whole body because the organism provides homeostatic mechanisms that mask disturbances in the administration of metals. Currently, interest is focused on the choice of the most suitable biological material which would allow assessment of changes in the human body. Hair and nails are excellent alternative materials which are partially independent of the influence of metabolic processes and regulatory mechanisms [12]. Additionally, hair and nails-based measurements may provide results from long exposure periods [13–15].

The aim of the study was to compare the levels of essential elements in patients with laryngeal cancer vis-à-vis healthy controls, using hair and nails as alternative materials. Furthermore, another aim was to demonstrate the advantages of these materials over serum in terms of their suitability for elemental analysis.

Materials and methods

Subjects

Samples of hair, nails and serum were collected from 73 healthy volunteers (51 women and 22 men), of which 20.5% were aged between 20 and 39 years old, 24.7% between 40 and 59 years old, and 54.8% between 60 and 65 years old, and 66 patients diagnosed with cancer of the larynx (5 women and 61 men). Patients were from the Otolaryngology and Laryngological Oncology Clinic and Head and Neck Clinic of the Poznań University of Medical Sciences, Poland.

Characteristics of the cases are summarized in Table 1. All cases had squamous cell carcinoma (SCC). The tumors were mainly localized in the glottis and supraglottis. Tumor stages were classified using the UICC TNM Classification, which is an anatomically based system that records the primary and regional nodal extent of the tumor and the absence or presence of metastases. Each individual aspect of TNM is termed as a category:

- T stands for the primary tumor site;
- N stands for the regional lymph node involvement;
- M stands for the presence or otherwise of distant metastatic spread.

Table 1
Characteristics of patients with larynx cancer.

Characteristics	Number of patients *n* (%)
Age	
40–59 years	36 (54.5%)
60–65 years	30 (45.5%)
TNM classification	
T2N1M0	20 (30.3%)
T3N1M0	15 (22.7%)
T4N2M0	31 (47%)
Histologic grade	
G1 (well differentiated SCC)	20 (30.3%)
G2 (moderately differentiated SCC)	30 (45.5%)
G3 (poorly differentiated SCC)	16 (24.2%)

When considering histologic grade, tumors are traditionally graded into well, moderately and poorly differentiated SCC [16].

All patients underwent routine blood tests (full blood count FBC, activated partial thromboplastin time aPTT, prothrombin time PT and international normalized ratio INR, total protein TP, erythrocyte sedimentation rate ESR, creatinine, fibrinogen, total bilirubin, blood urea nitrogen BUN, glucose, hepatitis B surface antigen HBsAg, total cholesterol, LDL, HDL, total plasma triglyceride, sodium Na, potassium K, chloride Cl, iron Fe, ALT, and AST). The results were not presented here, but general trends were evaluated. In most cases, the levels of hemoglobin, erythrocytes, iron in whole blood and proteins were decreased. It should be emphasized that the blood parameters are also affected by factors that predispose to cancer of the larynx, such as cigarette smoking and alcoholism. More details regarding diet and lifestyle habits can be found elsewhere [17].

The protocol of the study was approved by Bioethics Committee of the Poznań University of Medical Sciences, Poland.

Preparation of samples

Samples from patients were collected at least one year after diagnosis of cancer. The sample collection protocol and washing procedures were as described previously [18]. About 4–5 cm sections of hair were collected from the occipital region as near as possible to the scalp with the use of ceramic scissors. If the hair were dyed, the samples were taken from the roots at least two weeks after coloring. The samples were cut into smaller pieces (4–5 mm). The fingernails samples were collected using ceramic scissors. Next, the hair and nails samples were washed with deionized water (2 mL, vortex mixed for 3 min, twice), 1% Triton (2 mL, vortex mixed for 20 min), deionized water (2 mL, vortex mixed for 3 min, repeated until the detergent was removed), and methanol (2 mL, vortex mixed for 5 min, ultrasonicated for 15 min). The cleaned samples were dried at 80 °C for at least 6 h and then stored in desiccators.

The blood samples were collected from the median cubital vein without anticoagulants and placed in vacutainer tubes. Serum was separated by centrifugation at 14 000 rpm for 10 min. The serum samples were stored at −20 °C until digestion.

A microwave digestion system (MARS 5X CEM, Matthews, USA) was used for the decomposition of samples. Microwave digestion was performed under optimized conditions. Temperature and pressure were controlled during the heating program. The hair (0.2 g) and nails (0.07–0.1 g) samples were soaked in 7 mL of concentrated HNO_3 (which was added to the digestion vessel containing the sample). The samples were left overnight before the digestion process took place. In the case of serum, 7 mL of concentrated HNO_3 was added to 0.5 mL of serum samples directly before digestion. After the process, the digests were quantitatively transferred to 25 mL volumetric flasks and diluted with deionized water. The samples were stored in polyethylene vessels in a refrigerator.