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Serum and whole blood Zn, Cu and Mn profiles and their relation to redox status in lung cancer patients

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ABSTRACT

Disturbed redox status may be critical to lung cancerogenesis, however little research has been conducted on general changes in total redox status in lung cancer. Levels and activities of antioxidants, especially enzymatic ones, are related to trace element concentration. Trace element status is often disturbed in cancers, however no studies concerning the association between redox and trace element status have been performed for lung cancer. We hypothesized that disturbed redox status in lung cancer patients is partially determined by trace elements while their distribution amongst blood compartments may differ compared to healthy subjects.

Blood samples from lung cancer patients (n = 44) and control subjects (n = 44) were collected to assess redox and trace element status. Serum and whole blood Cu and Mn levels were determined with GF-AAS, and Zn–with F-AAS. In serum the total antioxidant status (TAS) was determined with the commercial kit TAS (Randox, UK), total oxidant status (TOS) was determined based on the method developed by Erel and the oxidative stress index (OSI) was calculated. Total protein (T-Prot), albumin (Alb), uric acid (UA) and total bilirubin (T-Bil) concentrations were measured with an auto-analyser (Konelab 20i, Thermoscientific, USA), SOD and CAT activity – with commercially available kits (Cayman, USA).

The level of TAS, T-Prot, Alb, T-Bil, the activity of SOD, the concentration of whole blood Mn as well as serum and whole blood Zn were lower while TOS, OSI, serum Cu levels and serum Cu:Zn ratios were higher in lung cancer patients compared to the control group. In the lung cancer group TAS correlated positively with Alb and UA, serum Zn and negatively with whole blood Mn. Additionally, SOD positively correlated with the whole blood Mn and Cu:Zn ratio, while CAT – negatively with the whole blood Cu:Zn ratio. In the lung cancer subgroup at clinical stage I–II, TOS additionally negatively correlated with whole blood Zn, and CAT negatively with serum Cu and Cu:Zn ratio. In advanced lung cancer, we found a positive correlation between TAS and serum Zn, and a negative one – with serum Cu:Zn ratio. We observed a similar correlation between endogenous non-enzymatic antioxidants and TAS in the control group, however considerably fewer correlations between trace elements and antioxidants were observed.

This study supports the hypothesis that disturbed redox status in lung cancer patients is linked with alterations in trace element status regarding Zn, Mn and Cu. Moreover, the type of biological fluid influences both – alterations in the metal profile and relationships with redox status parameters.

1. Introduction

The etiopathogenesis of lung cancer is multifactorial, however imbalanced pro/antioxidant status might be critical due to the well-known role of smoking in the development of this disease [1]. Despite this, only a few studies have been conducted on general changes in total redox status in lung cancer, and those performed have indicated a decrease in total antioxidant status, but not clearly explained the factors influencing the depletion [2]. Exogenous antioxidants contribute to defense against oxidative damage [3], however endogenous ones, among them the enzymes superoxide dismutase and catalase, are essential by virtue of the disproportionate reactions of their substrates –

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superoxide radical and hydrogen peroxide respectively − and are not consumed stoichiometrically. Therefore they present an enormous theoretical advantage over exogenous antioxidants, and are of major significance in the free-radical theory of cancer [4–6]. The content and activity of enzymatic antioxidants are both related to trace element concentrations. Zn, Cu and Mn are necessary for the proper activity of SOD due to their integral role as a cofactors or ions stabilizing the molecular structure [7]. However these metals function as antioxidants by further mechanisms e.g.: the inhibition of NADPH oxidase, the generation of metallothionein synthesis, and many more [7,8]. Moreover, Cu has differential redox activities related to oxidation state [9]. Concentrations of these metals change under cancerogenesis and are related to cancer stage and localization. In general, blood Zn and Mn levels are decreased, while Cu level is increased in cancer patients, however different artifacts including the type of biological material used for analysis, may make changes in the trends observed [10–12]. Alterations in serum/plasma trace elements have been shown in patients with lung cancer [13,14], however no studies concerning the evaluation of whole blood trace metal levels have been performed. In this study we used serum and compared it with whole blood to find: (1) biological material which better reflects changes in trace element status and associations with redox status, and (2) differences in trace element perturbations between these two types of biological material. Whole blood profile of trace elements, which accommodates their incorporation into blood cells, precisely reveals the long-term status of metals in the body and therefore might be more relevant to pathological conditions, including cancer [15]. In contrast to whole blood, serum/plasma concentration of minerals reflects short-term changes and may be affected by current dietary intake. Moreover, cancerogenesis may lead to a shift between compartments of the blood, and serum/plasma determination may therefore falsify the data on trace element loss [15,16].

In this study, to address whether there is any connection between redox and trace element status, total redox status, main endogenous antioxidants trace elements concentrations were assessed in both lung cancer patients and healthy subjects, and the relationships between these parameters and the clinical stages of lung cancer were established.

2. Subjects and methods

Eighty-eight subjects were recruited to this study. Lung cancer patients, (n = 44) were recruited from the Lower Silesian Centre of Lung Diseases. The control group (n = 44) consisted of healthy people recruited from Wroclaw 3rd Age Universities and public offices. Exclusion criteria for the control group were as follows: cancers, metabolic disturbances, other pro-inflammatory diseases, and mental diseases. The patient and control groups were sex and age-matched. The majority of lung cancer patients (85,7%) were suffering from non-small cell lung cancer (NSCLC). Patients were diagnosed with different clinical stages of disease − more than half at stages III and IV (NSCLC). Due to the assessment of trace mineral status, to which dietary factors are crucial, subjects were assessed in terms of dietary intake. Detailed characteristics of the lung cancer and control groups are presented in Table 1.

3. Methods

3.1. Assessment of nutrient intakes

Dietary data were gathered using three 24-h dietary recalls by a trained interviewer. In the case of lung cancer patients this took place on the first day of admission to hospital. To assess information about the portion size of food products, the “Album of Photographs of Food Products and Dishes” (National Food and Nutrition Institute, Warsaw, Poland) was used [17]. All dietary recalls were analyzed using Dieta 5.0 (National Food and Nutrition Institute, Warsaw, Poland).

3.2. Blood collection and preparation

The day after patient admission to hospital, blood samples (8 ml) were collected. Part of the whole blood (2 ml) was frozen unchanged, while serum was separated from 6 ml of blood. All material was stored at −80° C until analysis. Control group subjects' blood samples were collected and prepared in the same way.The study protocol was approved by the Ethics Commission of Wroclaw Medical University (approval no. 540/2013), and the study was conducted according to the principles expressed in the Declaration of Helsinki. All participants provided written consent for participation in the study.

3.3. Determination of trace element status

Zn, Mn and Cu concentration in serum and whole blood was determined with atomic absorption spectrometry (AAS).

3.3.1. Serum

Flame atomization was used for serum Zn concentrations while serum Cu and Mn levels were measured with graphite furnace atomization, using a ZEEnit 700P spectrometer (Analytik Jena, Germany). Concentrations of Zn and Cu were directly measured in serum. In the case of Mn, 0.2 ml of serum was deproteinated with 0.1 ml of HNO_3, 69–70%, before analysis. Then supernates were centrifuged at 7000 RPM for 10 min and serum Mn concentrations were measured. Accuracy of the method was measured with certified reference material (Seronorm TM Trace Elements Serum L-2, Sero AS, Norway). Analytical values of Zn, Mn and Cu in the reference material were: 2520 μg/L, 19.9 μg/L and 2887 μg/L, respectively. Mean accuracy (n = 6) was as follows: 113.0% (Zn), 93.1% (Mn) and 94.0% (Cu).

3.3.2. Whole blood

Whole blood preparation (0.5 ml) was performed twice by microwave −technique wet mineralization in a closed system using MLS 1200 Mega (Milestone, Italy), with mixture 1:5 of H_2O_2, 30% (Sigma-Aldrich, USA) and HNO_3, 69–70% (Baker Chemicals, USA). Flame atomization was used for the determination of Zn concentration and graphite furnace atomization for the determination of Mn and Cu concentration, using a PinAAcle™ 900T spectrometer (Perkin Elmer, USA). Accuracy of the method was measured with certified reference material (Seronorm TM Trace Elements Whole Blood L-3, Sero AS, Norway). Analytical values of Zn, Mn and Cu in the reference material were: 8.97 mg/L, 47.3 μg/L and 2.47 mg/L, respectively. Mean accuracy (n = 6) was as follows: 98,3% (Zn), 105.9% (Mn) and 91.6% (Cu).

For determination of serum and whole blood Zn concentration, a zinc hollow cathode lamp at 213.86 nm wavelength with a slit width 0.7 nm and air (10 l/min.)-acetylene (2.5 l/min.) burner was used.

Data on GF-AAS technique for serum Cu and Mn determination were as follows: 324.75 nm and 279.48 nm (wavelength of hollow cathode lamps, respectively), 1300° C (pyrolysis temperature for both) 2200° C (atomization temperature for both), 0.7 nm and 0.2 nm (slit width, respectively), argon (inert gas), 5 μl of palladium-magnesium nitrate modifier (for both), 20 μl (sample volume for both). In the case of whole blood all methodological data for Cu and Mn were the same, except the atomization temperature for serum Mn: 2000 °C.

3.4. Biochemical analysis

3.4.1. Determination of total antioxidant status (TAS)

Total antioxidant status (TAS) was measured in serum by the generation of 2,2′-azino-di-(3-ethylbenzthiazoline sulphonate) (ATBS) radical cation using the commercial kit TAS (Randox Laboratories, UK) on an auto-analyser (Konelab 20i ThermoScientific, USA).

3.4.2. Determination of total oxidant status (TOS)

Total oxidant status (TOS) was measured as described by Erel [18].