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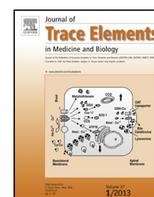

CLINICAL STUDIES

The influence of macro and trace elements on sperm quality

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ABSTRACT

The aim of this study was to examine the association between combined concentrations of macro and trace elements and markers of oxidative stress and antioxidative defense system function together with selected cytokine levels. Based on the combined medians of the seminal plasma levels of calcium, magnesium, zinc, copper, iron, and selenium, the study subjects (88 fertile male volunteers) were divided into the following two subgroups: the Me-L group (low level of metals) and the Me-H group (high level of metals). There was a tendency toward reduced motility in the Me-H group compared to that in the Me-L group. The total protein, albumin, and total oxidation status (TOS) levels were significantly higher in the Me-H group than in the Me-L group. The total superoxide dismutase (SOD), Mn-SOD, and CuZn-SOD, activity in spermatozoa were significantly lower in the Me-H group than in the Me-L group. In seminal plasma, the Mn-SOD activity was significantly higher in the Me-H group, whereas the CuZn-SOD activity was significantly lower. Additionally, the activity levels of glutathione peroxidase (GPx) and glutathione-S-transferase (GST) were lower in the Me-H group. The medians of IL-1β, IL-10, and IL-12 were significantly higher in the Me-H group than in the Me-L group, whereas the medians of IL-2, IL-5, and IL-13 were significantly lower. Higher levels of macro and trace elements in the seminal plasma of fertile males may be associated with decreased motility. Higher levels of the examined metals are associated with elevated oxidative stress accompanied by decreased activities of some of the antioxidant enzymes and increased pro-inflammatory cytokine levels.

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Introduction

Human sperm is very rich in macro and trace elements, including calcium (Ca), magnesium (Mg), zinc (Zn), iron (Fe), copper (Cu), and selenium (Se). The influence of these metals on semen quality has a significant effect on male fertility.

The prostate, seminal vesicles, and epididymis are very rich in Ca, and a number of studies have investigated the association between Ca and male subfertility [1]. Ca is the trigger of the acrosome reaction in mammalian spermatozoa, and there is substantial evidence that Ca is differentially involved in sperm motility, depending on the stage of sperm maturation [2,3]. Additionally, Mg, which is present in semen at a high concentration, is a crucial element in cell physiology [4]. Mg might play a role in spermatogenesis and sperm motility, and it is a marker of the secretions of the seminal vesicles and acts as an intracellular Ca antagonist [5].

Additionally, Zn is crucial to the quality of sperm and acts as a cofactor for most enzymatic reactions. The concentration of Zn is high in the prostate compared to that in other tissues and body fluids. Seminal plasma Zn levels have been positively associated with sperm concentration and motility [1,6]. After Zn, Fe is the second most important trace metal in many enzymes and metalloprotein compounds. Fe participates in oxygenation and reduction processes, and Fe ions are vital for normal cell growth and development, DNA synthesis, and electron transfer; however, Fe ions are toxic when present in excess [7]. Fe might induce lipid peroxidation in semen resulting in inhibition of sperm motility [8]. Cu, in its ionic form, might be toxic to a variety of cells, including human spermatozoa. Most seminal plasma Cu originates from the prostate as zinc [9]. Cu is an important element for numerous metalloenzymes and metalloproteins such as cytochrome oxidase, lysine oxidase, dopamine-β-hydroxylase, and ceruloplasmin, which are involved in energy or antioxidant metabolism [10]. In addition to being a cofactor of superoxide dismutase (CuZn-SOD), Cu with Zn prevents deleterious effects of reactive oxygen species on spermatozoa [11]. Another trace metal that exerts

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antioxidant effects is Se, which is incorporated into glutathione peroxidases (GPxs), a family of antioxidant selenoproteins found in the spermatozoa midpiece [12]. Se is an essential element for normal testicular development, spermatogenesis, and spermatozoa motility and function [13]. The best-characterized spermatozoa effects of Se deficiency are loss of motility, breakage at the midpiece level [13,14] and an increased incidence of sperm-shape abnormalities, predominantly of the sperm head [14].

Oxidative stress (OS) has been proposed as one of the most important factors affecting male fertility [15]. Reactive oxygen species generation is controlled by a complex range of cytokines, chemokines and other biologically active molecules in seminal fluid that protect sperm against the negative effects of OS [16].

In this study, we investigated selected macro and trace elements from among these active molecules. The data on their influence on semen quality and male fertility are inconclusive. Some authors suggest that macro and trace elements have a beneficial effect on fertility, whereas others report no significant effects or adverse effects from macro and trace element supplementation. These discrepancies might be because of very complex interactions among these elements. They might increase radical processes in or protect against ROS in semen. We simultaneously investigated a group of macro or trace elements. The aim of this study was to examine the association between combined concentrations of macro and trace elements and markers of oxidative stress and the antioxidative defense system function and levels of selected cytokines.

Materials and methods

Study population

The study population consisted of 88 healthy, non-smoking, fertile men from the southern region of Poland. The patients were drug tested, and no drug consumption (including antioxidant medications) was reported at the time of the study. The semen specimens of the participants were normal according to the WHO standards [17]. The concentrations of metals (Ca, Mg, Zn, Fe, Cu and Se) in semen were normalized and summed. Median of the obtained values was used to make a division of the study population into the following two groups: a low level of metals group (Me-L, $n = 44$) and a high level of metals group (Me-H, $n = 44$).

Samples collection

The semen specimens were collected in the morning on the identical day before the first meal. The semen samples (2–6 mL) were collected by masturbation, at home or at laboratory research facilities, after at least 3 days of sexual abstinence (the number of days elapsed since his last ejaculation was recorded for each volunteer).

Semen analysis

The semen specimens were analyzed according to WHO standards [17], including assessment of the seminal volume, sperm cell density, total sperm cell count, motility, and supravital eosin staining (for the percentage of live spermatozoa). The sperm morphology was examined after Papanicolaou staining. After liquefaction, the semen samples (1.5 mL) were centrifuged at 6000 × *g* for 10 min to separate the spermatozoa from the seminal plasma. The seminal plasma was transferred to fresh tubes and stored at −75 °C until required for biochemical and lead analyses. Additionally, a 10% spermatozoa lysate in bi-distilled water was prepared.

Determination of magnesium

The seminal plasma magnesium was determined using a Unicam flame atomic absorption spectrometer. This method involves diluting seminal plasma with a 0.2% solution of cesium chloride (CsCl), followed by determining the magnesium in solution by measuring the absorbance at a wavelength of 285.2 nm. The concentrations are expressed as mg/dL.

Determination of calcium

The seminal plasma calcium concentration was determined by the method of [18] using a biochemical analyzer method based on the reaction with o-cresolphthalein at pH > 7. The color intensity is directly proportional to the calcium concentration, which was expressed in mg/dL.

Determination of iron

The seminal plasma iron concentration was determined photometrically by the method of [19]. In this method, ascorbate reduces Fe^{3+} ions to Fe^{2+} ions, which react with ferrozine to form a colored complex. The color intensity is directly proportional to the iron concentration, which is expressed in μg/dL.

Determination of zinc

The concentration of seminal plasma zinc (ZnS) was measured by atomic absorption spectrophotometry using Unicam 929 and 939OZ Atomic Absorption Spectrometers with GF90 and GF90Z, respectively, at a wavelength of 213 nm [20]. Before each assay, 1500 μL of deionized water was added to 30 μL of the sample and mixed thoroughly before the analysis. The calibration curve was prepared according to Merck standards. The spectrophotometer was calibrated after each series of assays and the calibration curve. For the internal control, we used a Zn concentration of 0.1 mg/mL, which is a certified Merck standard control. The data are shown in mg/L.

Determination of copper

The concentration of seminal plasma copper (CuS) was measured by atomic absorption spectrophotometry using Unicam 929 and 939OZ Atomic Absorption Spectrometers with GF90 and GF90Z, respectively, at a wavelength of 324.8 nm [20]. Before each assay, 500 μL of deionized water was added to 50 μL of sample and mixed thoroughly before the analysis. The calibration curve was prepared according to Merck standards. The spectrophotometer was calibrated after each series of assays and the calibration curve. For the internal control, we used a Cu concentration of 10 mg/L which is a certified Merck control. The data are shown in μg/dL.

Determination of selenium

The concentration of seminal plasma was determined by a flameless method using Unicam 929 and 939OZ spectrophotometers. The calibration curve was prepared using Nycomed® standards. Certified Nycomed® controls (containing 78.0 and 11.4 μg/dm^3 of Se) were used to perform the internal control. The data are shown in μg/dL [21].

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