ANTI-AGE ANTIBODIES AND SERUM CONCENTRATION OF ZINC, COPPER AND SELENIUM IN PATIENTS WITH OCCUPATIONAL VEGETATIVE POLYNEUROPATHY OF UPPER LIMBS

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Summary

The aim of this study was to investigate the level of anti-AGE antibodies (AGE Abs) and serum concentration of zinc (Zn), copper (Cu) and selenium (Se) in the blood of 11 patients (group 1-G1, mean age 52.45±3.59 years), with occupational vegetative polyneuropathy of upper limbs (OVPUL) and 9 control patients (control group-G2, mean age 51.5±2.18 years). AGE Abs were assessed with direct enzyme-linked immunosorbent assay (ELISA). Atomic-absorption spectrometry was used to determine Zn, Cu and Se concentrations in the human’s sera.

The level of AGE Abs in the patients with OVPUL was significantly higher than in controls (p = 0.008). The patients with OVPUL had a significantly decreased concentration of serum Se, as compared to control group (p = 0.001). There were no differences in serum concentration of Zn and Cu in all groups.

Our data shows a negative correlation between increased levels of AGE Abs and the serum levels of Se in patients with OVPUL (r = -0.365, p = 0.047). Our study confirms that serum level of Zn, Cu and Se can be critical for maintenance of anti-oxidative events and propose that these trace elements may be important physiopathologically in occupational vegetative polyneuropathy of upper limbs.

Key words: anti-AGE antibodies, occupational vegetative polyneuropathy of upper limbs, zinc, copper, selenium

Introduction

Occupational vegetative polyneuropathy of upper limbs (OVPUL) results from systematic overstrain, microtraumas or pressure exerted on the structures of the hands. The disease is characterized by local pain in the distal part of the upper extremities and thermoregulatory, vasomotor and neurodystrophical disturbances [1]. Vascular microtraumas that result from repeated monotonous movements of the upper limbs lead to structural alteration in the vascular wall and decreased blood flow in the peripheral vessels, which causes hypoxia and pain.

Advanced glycation end-products (AGEs) form as a result of non-enzymatic reactions, in which glucose forms adducts with proteins, lipids and nucleic acids. AGEs accumulate naturally as a result of chronological ageing, but this process is greatly accelerated under conditions of hyperglycemia and oxidative stress [2].
AGEs act directly, as well as via specific receptors to alter the function of many intra- and extracellular proteins, including antioxidant and metabolic enzymes [3]. AGEs can induce oxidative stress [4]. The formation of AGEs cross-links changes the molecules of long-lived structures by intermolecular cross-linking and side-chain modifications, thus changing their antigenicity [5]. The immune system registers these modified own structures as non-self and reacts with an antibody production. Glycated proteins form common immunological epitopes, which result in the formation of populations of anti-AGE antibodies (AGE Abs). It has been established that AGEs possess antigenic similarity, regardless of the protein, on which they are formed [6].

Recent studies on the role of trace elements in the pathogenesis of several diseases are gaining importance. Of the various minerals of biochemical importance, zinc, copper and selenium play a pivotal role in the oxidant/antioxidant mechanism, the imbalance of which leads to increased susceptibility to oxidative tissue damage, thereby leading to disease pathogenesis in conditions such as diabetes mellitus, cancer, hypertension, etc. [7]. Zinc (Zn) and copper (Cu) are essential cofactors in copper/zinc superoxide dismutase (SOD-1) - an antioxidant enzyme, one of the first-line defence enzymes in scavenging reactive oxygen species [8], while selenium (Se) is a component of glutathione peroxidase (GSH-Px) one of the most important enzymes in the antioxidant protection of the organism. Se is known as the active center of GSH-Px, and is related to the reduction of hydrogen peroxide (H$_2$O$_2$) concentration in living organisms. In the Se-deficient condition, the GSH-Px activity is decreased [9]. Therefore, it is predictable that oxidative stress due to H$_2$O$_2$ occurs.

AGE Abs and their connection with markers of oxidative stress have not as yet been examined in OVPUL. We therefore investigated the presence of antibodies against AGEs and concentration of Zn, Cu and Se in sera of patients with OVPUL.

**Materials/Patients and Methods**

**Subjects**
We studied 11 individuals with the OVPUL, compared to a healthy normal control group of 9 sex- and age-matched individuals. Patient demographics are shown in Table 1. We define “age-matched” as groups of patients whose average age ranged between 38 and 59 years. All sera samples were stored at -70°C before use.

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<thead>
<tr>
<th>Table 1. Patient Demographics</th>
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<tr>
<td><strong>Subjects</strong></td>
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<tr>
<td>Patients with OVPUL - G1</td>
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<td>HNC - G2</td>
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N-number, M-male, F-female, HNC healthy normal control group, OVPUL - occupation vegetative polyneuropathy of upper limbs

**Assay of concentration of trace Elements**
Zn, Cu and Se concentrations in the subjects’ sera were analyzed by flame atomic-absorption spectrophotometry, using Perkin-Elmer, Model-Analyst 300.

**Glycation of KLH for antigen**
Keyhole Limpets Hemocyanin (KLH) (Sigma, 20mg/ml) was glycated *in vitro* with 3.33 M glucose in 0.4 M phosphate buffer, pH 7.5 with preservative 0.04% sodium azide, at 37°C, for 12 weeks. The formation of advanced glycated end-products of KLH (AGE-KLH) was quantified by measuring the fluorescence at 360/440 nm
excitation/emission. The obtained AGE-KLH was used as antigen in direct ELISA.

**ELISA for determination of anti-AGE antibodies**

The serum levels of anti-AGE (IgG and IgM) antibodies were measured by a home-made ELISA. 96-well microtiter plates (MICROLON, U-bottom, high binding, Greiner Bio One, Frickenhausen Germany) were coated with AGE-KLH by adding 100 µl of a solution of (10 µg/mL dissolved in 0.05 M carbonate buffer, pH 9.6) to each well and incubating for 2 hr at 37°C and overnight at 4°C. Wells were washed with a solution of PBS, containing 0.05% Tween 20 (PBS-Tween) and then blocked by incubation for 1 hr with 1% bovine serum PBS-Tween, after which 100 µl of patient sera, diluted 1:5 in PBS-Tween was added. The plates were incubated for 1 hr at 37°C. The wells were then washed with PBS-Tween, incubated with a peroxidase-linked anti-human IgG and IgM (Bul Bio, National Center for Infectious and Parasitic Diseases, Sofia, Bulgaria) diluted at 1:6 400 for IgG and 1:12 800 for IgM in 1% human serum albumin in PBS-Tween, and after the washings reacted with o-phenylenediamine plus 0.1% H₂O₂ as colorimetric substrate. The reaction was terminated by 50 µl 8M H₂SO₄ and the absorbance was read at 492 nm on automatic micro-ELISA plate reader. Each sample was analysed three times and the mean value was used for statistical analysis.

**Statistical Analysis**

Differences in anti-AGE IgG and IgM autoantibodies and serum concentration of Zn, Cu and Se between the groups were analysed for statistical significance (P < 0.05) with one-way analysis of variance (ANOVA) and multiple comparison test - Least Significant Difference (LSD method) using the statistical package SPSS, v. 13 (SPSS Inc., Chicago, IL, USA).

**Results**

The results of the ELISA used for measuring AGE Abs levels showed significant difference (p=0.008) between patients with OVPUL -G1 and their age-matched controls G2. (Fig. 1)

The data for serum concentration of Zn and Cu in the two groups we investigated are summarized in Fig.2. The serum concentration of Zn was: G1 14.31±0.54, G2 13.7 ±0.42 (µmol/l), and Cu G1 19.0±1.24, G2 21.87±2.85 (µmol/l). There were no significant differences between patients with OVPUL (G1) and the control group (G2).

The patients with OVPUL had a significantly decreased concentration of serum Se G1 (777.07±48.88), as compared to the control group G2 (1074.27±14.90, p=0.001), (nmol/l). (Fig. 3)

Our data showed a negative correlation between increased levels of AGE Abs and the serum levels of Se in patients with OVPUL (r =-0.365, p =0.047).

**Figure 1.** Levels of anti-AGE antibodies in patients with OVPUL -G1 and their age-matched controls G2. Data are reported as mean±SEM.

**Figure 2.** Serum concentration of Zn and Cu in patients with OVPUL G1 and control group G2. Data are reported as mean±SEM.
Discussion

The etiology and disease mechanisms of OVPUL are not completely clear. A number of studies suggest that systematic overstrain and lack of restorative periods cause tiredness and exhaustion of the muscles and joints and affect the tissue metabolism, which results in oxidative stress. Oxidative stress is one of the major factors in the pathogenesis of OVPUL, because oxidation-damaged cell structures are more susceptible to the influence of free glucose and non-enzymatic formation of early and late products of glycation.

Anti-AGE antibodies are found in the serum of healthy human subjects as part of the homeostatic mechanism, which clears altered structures via in situ destruction or via opsonization. Excessive accumulation of AGEs with age and in pathology appears to correlate with elevated levels of AGE Abs [10]. AGEs accumulate in the vessel wall, where they may perturb cell structure and function. AGEs have been implicated in both microvascular and macrovascular complications of diabetes [11].

In our study, AGE Abs were present in all the samples investigated. We observed significantly higher levels of AGE Abs in patients with OVPUL, as compared to the control group. To address the connection between AGE Abs and the oxidative status of the investigated patients, we tested the serum levels of Zn, Cu and Se as markers of oxidative stress. Transition metals such as zinc, copper and selenium are cofactors for intra- and extracellular oxidases, oxygenases and dismutases. These are preventive antioxidants, which eliminate species that are involved in radical chain processes [12]. In addition to its antioxidant effects, selenium has been reported to have strong anti-diabetic and insulin-mimetic effects [13,14,15]. In the patients with OVPUL, we found a significantly decreased Se concentration of serum. Selenium is especially important in antioxidant enzymatic defences regarding its structural function in the active site of selenoenzyme GPx. As Se is used to maintain GPx synthesis, the decreased Se level is most probably due to the plasma GPx activity depletion under conditions of elevated generation of free radicals. Our data showed a negative correlation between increased levels of AGE Abs and the serum levels of Se in patients with OVPUL. This connection is very important, because selenium has insulin-mimetic effects. These effects are mediated through the activation of key proteins involved in the insulin-signal cascade [14] and Se-deficiency may evoke glucose intolerance associated with an increase of gluconeogenesis [16]. A direct consequence of insulin resistance is accumulation of AGEs in all structures, particularly in the vasculature.

Conclusions

AGE Abs and their connection with oxidative stress markers in OVPUL still have not been thoroughly investigated. Based on our findings, we assume that the early administration of consistent Se amounts to improve the antioxidant defense should be of great interest in restoring oxidative tissue alterations and the development of OVPUL.

References


