SERUM COPPER, ZINC AND SELENIUM LEVELS IN RHEUMATOID ARTHRITIS

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SUMMARY

The objective of this study was to measure the alterations in some important serum trace elements, including selenium (Se), zinc (Zn), copper (Cu), in patients with rheumatoid arthritis (RA). In the study, serum trace elements were determined by using Atomic Absorption Spectrophotometer in sera of patients with RA (n=32), and statistically compared with healthy individuals (n=52). Serum Cu concentration was found significantly higher in patients group than those of healthy individuals (26,06±4,.71 and 17,74±3,30 µmol/L, respectively) (p<0,001). In contrary, Se and Zn levels were lower in patients (0,18±0,05 µmol/L and 6,58±3,14 µmol/L, respectively) according to the healthy subjects (0,21±0,06 µmol/L and 11.44±6,00 µmol/L, respectively).

Our results showed that serum trace elements (Se, Zn and Cu) concentrations are altered in RA patients. The changes may be a part of autoimmune process in organism induced by cytokines. In conclusion, we suggest that decrease in Zn and Se levels and elevation in Cu levels are probably responsive to inflammation and resulted from defence strategies of organism and mediated by the hormone-like substances.

Key Words: *Rheumatoid arthritis, selenium, zinc, copper*

ÖZET

ROMATOİD ARTRİT'DE SERUM BAKIR, ÇİNKO VE SELENYUM DÜZEYLERİ

Bu çalışmada, serum eser elementlerin önemlilerinden selenyum (Se), çinko (Zn) ve bakır (Cu), Atomik Absorbsiyon Spektrofotometre kullanılarak romatoid artritli (RA) hastaların (n=32) serumlarında belirlendi ve istatistiki olarak sağlıklı bireylerin değerleri ile (n=52) kıyaslandı.

Serum Cu düzeyleri hasta grupta 26,06±4,71 µmol/L olarak bulundu ve bu değer önemli ölçüde sağlıklı bireyler grubundan yüksekti (17,74±3,30 µmol/L) (p<0,001). Buna karşın, Se ve Zn düzeyleri hastalarda (Se:0,18±0,05 µmol/L ve Zn:6,58±3,14 µmol/L) sağlıklı bireylere göre daha düşük bulundu (Se:0.21±0.06 µmol/L ve Zn:11,.44±6,00 µmol/L).

Sonuçlarımız, RA'li hastalarda serumda eser elementlerinin (Se, Zn ve Cu) düzeylerinde değişim olduğunu gösterdi. Bu değişiklikler sitokinler tarafından organizmada uyarılan otoimmün sürecin bir parçası olabilir. Netice olarak, biz Zn ve Se düzeylerindeki düşme ve Cu düzeylerindeki artmanın muhtemel olarak yangıya yanıt olarak geliştiği ve organizmanın savunma stratejisinin bir sonucu olduğu ve hormon benzeri maddeler tarafından düzenlendiğini düşünmekteyiz.

Anahtar Kelimeler: *Romatoid artrit, Selenyum, Çinko, Bakır*

INTRODUCTION

The mechanism(s) by which cells play role in rhevmotoid arthritis (RA) RA pathogenesis has been the subject of intense research in recent years. The changes in trace element levels are part of immune system of organism and are induced by the hormone-like substances interleukin-1 (IL-1), tumour

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necrosis factor-a (TNF-a) and interleukin-6 (IL-6) (1,2). These substances are immunocytokines liberated dose-dependent mode, mostly by activated macrophages, in response to several stimuli, including exercise, trauma, stress, or infection (3). The most known changes in inflammation and infections are alterations in ferric ion (Fe), Zinc (Zn) and Copper (Cu) levels in sera associated with elevated levels of acute phase proteins, such as ceruloplasmin (4).

The onset of RA can vary among individual patients. The earliest alterations relate to the vasculature, with vascular congestion and even obliteration of small vessels by inflammatory cells and thrombi. In addition to this microvascular injury, hyperplasia and hypertrophy of the synovial lining cells and a modest perivascular accumulation of leukocytes are also typical findings. The chronic phase of RA is characterised grossly by edema and swelling of the synovium. While polymorphonuclear leukocytes are dominant in the synovium at onset, the extravascular lymphocytic infiltration becomes more abundant in the chronic phase. Here, it consists largely of CD4+ helper T cells (Th), which are in close apposition to antigen presenting cells. pathologically, this is quite similar to the characteristics of delayed-type hypersensitivity (Gell Coombs class type IV) (5).

Patients with RA can be classified into four classes according to the functional criteria described by Hochberg and his colleagues (6) as shown in the Table I.

In the treatment of RA, many drugs are available, such as gold compounds, non-steroidal anti-inflammatory drugs (NSAIDs), penicillamine, sulphasalasine, chloroquine, as well as, glucocorticoids and methotrexate as immunosuppressants (7). NSAIDs are blocking agents of the activity of cyclooxgenases (COX), namely COX-1 and COX-2, which catalyses the production of leukotriens as arachidonic acid metabolites. COX-2 is mainly present in inflammatory cells and inducible, while COX-1 is expressed in most tissues and constitutive enzyme. There are some novel drugs, such as nabumetone, show selective action on COX-2. The salicylates and paracetamol are commonly in use of RA treatment. The mode of action of sulphasalazine is not clearly known but there is evidence that it can scavenge toxic oxygen metabolites produced by neutrophils (7). Chloroquine, an anti-malarial drug, inhibits mitogen-induced lymphocyte proliferation and decreases leukocyte chemotaxis, lysosomal enzyme release and generation of toxic oxygen metabolites (7). Glucocorticoids are inhibitory agents for phospholipase A2 (PLA2), an enzyme catalyses the production of prostaglandins from arachidonic acid metabolites, and have a large scale effect on metabolism and immune system. Additionally, they also inhibit COX-2 enzyme (7). Methotrexate, an immunosuppressant and anti-neoplastic agent, is the main folate antagonist (8). All these drugs are used alone or in combination according to well-established clinical treatment protocols.

Table I: Functional Classification criteria of RA

Class 1:	Full adequacy in daily life
Class 2:	In spite of limitations in articles, normal activities can be done
Class 3:	Marked limitations are present, and the patient can not do his occupatinal work and his own care.
Class 4:	Disability or dependency on bed or wheel-chair, and he can not nearly almost do his own care

The purpose of the present study was to investigate the status of essential trace elements Se, Zn and Cu concentrations in patients with RA functionally classified into three groups (6) and in regard with their treatment.

MATERIALS AND METHODS

Totally 84 subjects were enrolled in the study, 32 patients (26 F, 6 M; mean of ages=47,69±14,20), and 52 healthy individuals (mean of ages = 37,85±10,95). All patients and voluntaries provided a written consent, confirming they accepted conditions of giving blood through vena brachialis and were informed about the whole experimental procedures. The patient group were diagnosed and classified by clinicians of Internal Medicine Clinic for the admission criteria. The group of healthy individuals was selected among healthy parents or siblings which had no any disease. Distribution of cases in regard with the activity and duration of the disease and their treatments were shown in the Table II, III and IV, respectively. After overnight fasting, total 10 ml venous blood was withdrawn. Then, 10 ml were transferred into tubes without any addition of anticoagulants and centrifuged for 15 min at a speed of 250xg. Sera were separated to determine Se, Cu and Zn levels.

Serum Se determination was performed by a SpectrAA 250 Plus Zeeman Atomic Absorption Spectrophotometer with a graphite furnace GTA-96 (Varian, Australia), with deuterium background correction. Varian hollow cathode lamps were employed at the 196-nm wavelength and 1.0-nm bandpass. Pyrolytically coated graphite tubes with pyrolytic graphite platforms (Varian, Australia) were used. Se concentration was determined by an internal standard addition method, as previously described (4). For the analysis of se, sera were diluted four times in nitric acid (v/v, 0.125%) with the addition of Triton-x100 (v/v, 0.05%). All the samples were run in duplicate, and 10µl of the sample in 2 ml of palladium-chloride and ascorbic acid (w/v, 2%) solution were assessed in the system. The lowest threshold Se detection of instrument was 10 µg/L. Accuracy and precision of procedure were regularly checked with commercial standards (Seronorm serum, Nycomed AS, Oslo, Norway).

Serum samples were diluted with deionised, distilled water for Cu and Zn measurements. Copper and Zn were determined by a SpectrAA 250 Plus Zeeman Atomic Absorption Spectrometer (Varian, Australia) with a deuterium background correction. Serum Cu and Zn values were expressed in µmol/L.

Table II: Distribution of cases in regard with sexes and the activity of disease

	Female (n/age means)	Male (n/age means)
Active RA	15 (45,67+11,02)	3 (48,20+16,20)
Remitional RA	11 (45,00+13,46)	3 (57,00+9,16)
Total	26 (46,85+14,90)	6 (51,33+10,98)

Table III: Distribution of cases in regard with duration of RA

Duration (years)	n
<5	20
5-10	8
10-15	2
>15	2

Table IV: Distribution of cases in regard with their treatment

Treatment	n
NSAID+Sulphasalasine	2
NSAID+Methotrexate+Corticosteroid	2
NSAID	7
NSAID+ Methotrexate+Corticosteroid+Sulphasalasine	1
NSAID+ Methotrexate	3
NSAID+Sulphosolozinc+Corticosteroid	6
Methotrexate+ Sulphasalasine	2
NSAID+ Corticosteroid	2
NSAID+ Corticosteroid+Chloroquine	3
NSAID+ Methotrexate+ Chloroquine	1
Salasopyrin+Gold	1
NSAID+ Corticosteroid+Gold	1
Methotrexate+Corticosteroid+Sulphasalasine	1
Total	32

Statistics were calculated with the SPSS for Windows Version 6.0 program. The mean values obtained in the two groups were compared by Student's t-test. The Pearson 's correlation test was used to evaluate the correlation amongst parameters. All results were expressed as mean values + SD; statistical significance was defined as p<0,05.

RESULTS

As shown in the Table V, when compared to controls, patients with RA had significantly lower levels of serum Se and Zn (p<0,01, p<0,001, respectively). However, serum Cu concentrations were higher in RA patients according to the control subjects (p<0,001). Additionally, in the comparison among the groups in regard with their treatment, it was surprisingly observed that the group receiving methotrexate had higher levels of zinc in their sera than the group not receiving the drug (p<0,.05) (Table VI. No difference was observed amongst the potients in different furctionol groups.

Table V: Serum Cu, Se and Zn levels in patients with RA and healthy subjects are shown

Trace Elements	Patients (n=32)	Controls (n=52)
Zn (µmol/L)	6,58±3,14**	11,44±6,00
Cu (µmol/L)	26,06±4,71**	17,74±3,301
Se (µmol/L)	0,18±0,05*	0,21±0,06

Results were expressed means±SDs in µmol/L (*:p<0,01, **:p<0,001).

DISCUSSION

Research effort has shifted from experiments to describe the changes in mineral metabolism associated with immune response to investigations of the mediators responsible. The observations that host products are released from stimulated leukocytes could induce metabolic changes similar to an acute-phase response revealed an endocrine role for the immune system. Characteristic changes in trace mineral metabolism are an integral part of the acute-phase response. The changes are usually reflected in decreased serum Zn and increased serum Cu concentrations (9). Although clear information is not present, decrease in Se levels in inflammation resembles zinc influence.

The role of certain inflammatory products in the regulation of the Zn balance has been well documented. Some interleukines released or secreted

Table VI: Serum Cu, Se and Zn levels in patients with RA in regard with their treatment are shown

	Zn		Cu		Se	
	–	+	–	+	–	+
Sulphosolozinc	6,76±3,06 (n=21)	6,27±3,37 (n=11)	26,22±4,55 (n=21)	25,59±5,34 (n=11)	0,18±0,05 (n=21)	0,18±0,04 (n=11)
Corticosteroids	6,53±3,21 (n=20)	6,70±3,37 (n=12)	26,22±4,87 (n=20)	25,91±4,71 (n=12)	0,18±0,05 (n=20)	0,17±0,04 (n=12)
Methotrexate	5,95±3,21 (n=22)	8,00±2,75 (n=10)	24,96±5,02 (n=22)	28,42±2,98 (n=10)	0,17±0,05 (n=22)	0,20±0,05 (n=10)
NSAIDs	6,32±3,52 (n=3)	6,62±3,21 (n=29)	24,65±7,07 (n=3)	26,22±4,55 (n=29)	0,18±0,01 (n=3)	0,18±0,05 (n=29)
Chloroquine	6,44±3,37 (n=28)	7,68±0,92 (n=4)	25,91±4,87 (n=28)	26,85±4,40 (n=4)	0,18±0,05 (n=28)	0,17±0,06 (n=4)

Results were expressed means±SDs in µmol/L (*:p<0,05) -:indicates the group without treatment, +:indicates with the group treatment

from leukocytes or activated phagocytes may cause to Zn deficiency by inhibiting its transport from plasma to the liver (10). Decreasing serum Zn levels apparently result from the synthesis of methallothionein (MT) in liver and other tissues. Methallothionein binds 7 g atoms of Zn per mol and serves to draw Zn away from free-circulating pools and it is induced by IL-1 in vivo (11).

Increased serum Cu is associated with Cp and induced by IL-1 (12). It was demonstrated that IL-1, but not TNF-a, induces hypercupremia when injected into the preoptic anterior hypothalamus (1). In our study we observed that Cu levels were significantly higher in patients' sera than in controls. Increased Cu levels may be attributable to inflammation associated with the disease.

As to Selenium, its importance in RA was indicated by some researchers (13). It has also been demonstrated that GSH-Px enzyme activity was lower in the patients with RA (14). However, it was not known whether the causes of this depletion were dependent on Se content or the other factors. Although it was indicated by some researchers that the reason of diminished levels of Se was nutritional deficiency in chronic inflammations (15), some other scientists suggested that nutritional deficiency was not a reason (16). The main source of Se is drink water, and although some RA patients are given Se, Se-dependent GSH-Px enzyme activation is not induced. Therefore, these findings indicate that decreased Se levels is a defence mechanism against inflammation. The decreased levels of Se in our patients may be attributed to redistribution from the plasma pool into the tissues as a defence mechanism that it modulates the effect of infection.

We conclude that serum essential trace elements Se, Zn and Cu concentrations were altered probably by the some immunocytokines as a host defence elements of organism during RA. Further investigations will be needed to study inununocytokines together with trace metals specially Se and antioxidant enzyme activities.

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