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ORIGINAL ARTICLE

Copper levels in patients with rheumatoid arthritis

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Abstract

Introduction. Rheumatoid arthritis (RA) is a chronic, autoimmune-based disease of the connective tissue with still unknown etiology. Numerous studies have indicated the association between Copper (Cu) and ceruloplasmin (Cp) concentrations and pathogenesis of RA.

Objective. To compare the concentrations of Cu and Cp in different biological samples and their correlation with the inflammatory process, between a group of patients with RA and a control group of healthy individuals.

Materials and Methods. The study enrolled 74 Caucasian patients (20 men and 54 women), aged 29–50 (mean age 39.8±6.1 years) diagnosed with RA. The control group consisted of 30 healthy Caucasian individuals. Copper levels were assessed by atomic absorption spectroscopy.

Results. Among RA patients the mean Cu level was significantly higher in serum and hair compartments and significantly lower in erythrocytes, compared with the control group ($p<0.01$). The Cp concentration was also higher in serum of RA patients ($p<0.001$). A statistically significant, positive correlation between the Cp serum concentration and the ESR values ($r_s = 0.38$; $p<0.007$) was found. No significant influence of pharmaceutical treatment (methotrexate, non-steroidal anti-inflammatory drugs, glucocorticoids, calcium, vitamin D_3 and sulphasalazine) on serum Cu was found.

Conclusions. It seems that the 'copper status' in patients with RA, based on the measurement of Cu and Cp levels in blood serum is correlated with presence of the inflammatory process. The hair could serve as a useful, additional diagnostic material. Some other factors, different from the applied treatment, can probably influence the Cu levels in patients with RA.

Key words

rheumatoid arthritis, copper, ceruloplasmin, inflammation

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, autoimmune-based disease of the connective tissue with still unknown etiology. A non-specific inflammation of the symmetric joints with their subsequent destruction, presence of extra-articular lesions and systemic complications are characteristic for RA. Many of its risk factors have not yet been established. Some researchers have focused on the role of such environmental factors as minerals and trace elements: Zinc (Zn), Copper (Cu), Selenium (Se), Magnesium (Mg), Iron (Fe), Manganese (Mn) [1, 2, 3], as well as smoking [4, 5] and the contribution of oxygen free radicals [6].

Copper is an environmental bioelement which plays a key role in the cell's physiology, as a cofactor or component of the enzymes, participating in anti-oxidative processes, or in detoxification of oxygen free radicals. Cu is also responsible for appropriate cartilage mineralization, formation of elastin and collagen structure [7], and creation of bony trabeculation structures, and collagen cross-linking [8, 9].

Approximately 90–95% of the total amount of this bioelement in blood serum is strongly protein-bound, mostly with α_2–globulin (ceruloplasmin) [5, 7]. Ceruloplasmin (Cp) performs some anti-oxidative actions, and represents one of the organism's protective mechanisms [7, 9]. Serum concentration of Cp increases in the presence of inflammation or infection [7], as a consequence of its enhanced synthesis. This is mostly due to the Cp production in hepatocytes, stimulated by proinflammatory interleukins, such as Il-1 and Il-6 [7]. In our previous study, we evaluated the levels of zinc present in three biological compartments (serum, erythrocytes and hair) in patients with RA [10].

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OBJECTIVES

The aim of this study was to compare the concentrations of Cu and Cp in different biological samples and their correlation with the inflammatory process, between a group of patients with RA and a control group of healthy individuals.

MATERIALS AND METHODS

The study enrolled 74 Caucasian patients (20 men and 54 women), aged 29–50 (mean age 39.8±6.1 years) diagnosed with RA. These individuals were consecutively attending two specialist Rheumatology Outpatient Clinics in Szczecin, Poland. The inclusion criteria applied to this group were as follows: diagnosis of RA based on the American College of Rheumatology criteria, such as joint involvement, results of serology testing, acute-phase reactants and duration of symptoms [11], no medications like diuretics, hypotensive agents, anticonvulsants, antibiotics, vitamin supplements containing the studied elements, or oral contraceptives

(females) taken for at least 6 months prior to the study, following a normal diet, no evident gastrointestinal or urinary disturbances or endocrine conditions, absence of concurrent inflammation, age less than 50, and regular menstruation (females), and informed consent to take part in the study. There were no patients treated with biological agents, only with disease modifying anti-rheumatic drugs and anti-inflammatory agents. The precise distribution of received treatment is described below.

RA patients were divided into 4 subgroups depending on the pharmacological treatment received during the last 6 months prior to the study:

- MTX = patients treated with methotrexate (MTX) and non-steroidal anti-inflammatory drugs (NSAIDs);
- MTX + GC = patients treated as above, also receiving glucocorticoids (GC) – prednisone at a dose not exceeding 12.5 mg daily;
- MTX + GC + Ca + D_3 = patients treated as above, also receiving calcium and vitamin D_3 (as a means of osteoporosis prevention);
- SAS = patients receiving sulphasalazine (SAS) and NSAIDs only.

Table 1 shows the distribution patterns of quantitative parameters in RA patients: mean disease duration, RA clinical activity – Ritchie articular index for joint tenderness [10], subjective joint pain score based on the Visual Analogue Scale (VAS), and haematology test results – erythrocyte sedimentation rate (ESR) and haemoglobin level (Hb).

Table 1. Study group characteristics (patients with RA, n = 74)

Parameter	Normality of distribution	Range	Median	Mean ± SD
Mean disease duration [years]	–	1.0–28.0	7.7	8.5±6.7
Ritchie articular index [points]	–	2.0–19.0	8.1	8.2±3.4
VAS [cm]	–	2.0–8.5	5.2	5.1±1.7
ESR [mm/h]	–	2.0–93.0	31.2	37.2±25.1
Hb [g/dL]	+	10.5–15.1	12.7	12.8±0.9
DAS28	–	2.6–6.5	5.1	4.9±0.9
Cp [g/L]	–	0.2–0.6	0.3	0.3±0.1

The control group consisted of 30 healthy Caucasian individuals (8 men and 22 women), aged 27–50 (mean age 38.2±8.3 years), admitted consecutively by two general practitioners practising in Szczecin, for reasons unrelated to RA. The inclusion criteria for this group were the same as for studied group, except diagnosis of RA.

Study design. All study participants had their medical history taken, underwent a physical examination, and had their copper levels measured: in the extracellular fluid (serum), in the intracellular compartment (erythrocytes and hair) and had their serum ceruloplasmine concentration measured.

The study was accepted by the Bioethical Commission (Ethics Board) of the Pomeranian Medical University in Szczecin for studies in human subjects. The study was conducted under KBN (Polish Scientific Research Committee) Grant No. 6 P05B 126 20. Written informed consent was obtained from all individuals from both study and control groups.

BIOCHEMICAL ANALYSIS

Serum and Erythrocyte Copper Concentrations. Copper levels in serum and in erythrocytes, as well as Cu content in hair, were measured by atomic absorption spectroscopy (AAS) using either an acetylene-air or acetylene-nitrous oxide flame. The serum end erythrocyte samples were prepared by mineralisation in a 5:2 mixture of ultrapure nitric acid and perchloric acid until achieving complete destruction of the organic matrix. Next, the solution was transferred quantitatively into a volumetric flask and filled up to the final volume with deionised water.

Serum Ceruloplasmin Level. Measurements of blood serum Cp level were conducted using a compact biochemical analyzer – COBAS MIRA. To measure the Cp concentration, the Unimate 3 CER reagent, and the turbidimetry method, measuring the degree of opacity at 340 nm wave length were used. This instrument was calibrated automatically. Similarly, the specimen collection and data transmission were performed automatically. For conducting this test, a blood serum sample of 600 µl was drawn (each time), and the laboratory reference range of normal Cp blood serum concentration was 0.20–0.60 g/L (20–60 mg/dL).

Hair Copper Content. The preparation of hair samples has been described previously by Mierzecki et al. [10]. The copper content was assessed by AAS using either an acetylene-air or acetylene-nitrous oxide flame. The measurements were conducted with a Pay-Unicam SP-9 spectophotometer interfaced with a computer.

The levels of Cu in serum, erythrocytes and hair were analysed at the Institute of Quantum Electronics, Military Technical Academy, in Warsaw, Poland. The Cp concentration was analysed at the Central Laboratory of the First Independent Public Clinical Hospital in Szczecin, Poland.

Statistical Analysis. Statistical analysis was performed using the StatSoft Polska v. 9.0 package (StatSoft Inc., Tulsa, Oklahoma, USA). The distribution type of the studied parameters within analysed groups was determined based on the Shapiro-Wilk test for normality (N), sample size (n), range of values (range), median (Me), mean ($\bar{x}$) and standard deviation (SD). The results from two independent groups were compared using the Student's *t*-test (when the distribution of a given parameter was not significantly different statistically from the normal distribution in any of the comparison groups) or the Mann-Whitney U test (when the distribution of a given parameter was significantly different statistically from the normal distribution in at least one of the groups being compared).

In order to determine a statistical relationship between two variables, either the Pearson's (linear) correlation coefficient (when both variables had normal distribution) or Spearman's rank correlation coefficient (with at least one variable not having the normal distribution) were used.

In all comparisons, the adopted significance threshold was p value ≤ 0.05.

RESULTS

In the study group of patients with RA, a statistically significant, higher serum Cu concentration, and higher hair content, as well as its significantly lower level in erythrocytes, were found, compared to the healthy individuals (control group) (Tab. 2). An average Cp level in serum of RA patients was statistically significantly higher then in the control group (0.29±0.10 g/L vs. 0.21±0.02 g/L; $p < 0.001$).

Simultaneously, despite showing various levels of this bioelement, depending on the treatment schedules, it was not found that the pharmacotherapy applied in the patients' group with RA significantly affected the levels of Cu, in any of the particular biological compartments (Tab. 3). Also, no correlation was demonstrated between the Cu concentration in serum, erythrocytes and hair, and the dose of MTX and GC (Tab. 4).

Table 2. Comparison of mean copper levels/content in serum, erythrocytes and hair of patients with RA versus the control group

Type of sample in which mean copper levels/content were assessed	RA patients (n=74)	Control group (n=30)	p
serum [µg/dL]	122.3±28.2	91.2±16.9	< 0.001
erythrocytes [µg/dL]	68.2±13.1	85.3±7.9	< 0.001
hair [µg/g d.h.m.[1]]	14.8±4.1	10.5±3.6	< 0.01

[1]dry hair mass

Table 3. Comparison of mean copper levels/content in patients with RA depending on the pharmacological treatment used

Type of sample in which mean copper levels/content were assessed	Treatment regimen				p
	MTX (n=19)	MTX+GC (n=17)	MTX+GC+ $Ca+D_3$ (n=20)	SAS (n=18)	
serum [µg/dL]	129.6±36.9	121.7±17.0	112.5±18.1	121.1±34.1	NS
erythrocytes [µg/dL]	64.4±16.4	70.8±9.6	64.5±16.3	69.5±11.9	NS
hair [µg/g d.h.m.[1]]	14.9±3.1	15.9±6.2	14.9±3.3	8.2±1.4	NS

[1]dry hair mass

Table 4. Correlation between the levels/content of copper in serum, erythrocytes, and hair and the dose of methotrexate (MTX) or prednisone (GC)

Type of sample in which mean copper levels/content were assessed	Spearman's rank correlation coefficient	
	MTX (n = 56)	GC (n = 37)
serum [µg/dL]	0.32	0.36
erythrocytes [µg/dL]	0.005	-0.07
hair [µg/g d.h.m.[1]]	0.06	0.33

[1]dry hair mass

In the analysis of the relations of Cu levels, and some of the active disease (RA) parameters, certain statistically significant, positive correlations between the serum Cu concentration and the values of ESR, DAS28 and Ritchie articular index, and the Cp serum concentration were found. On the other hand, negative correlations of the above RA parameters with the haemoglobin level were found (Tab. 5). In like manner, positive correlations between the Cu concentration in erythrocytes and haemoglobin level were demonstrated. The remaining associations analysed in this study were not statistically significant. In addition,

Table 5. The values of Spearman's rank correlation coefficients (r_s) or Pearson's (linear) correlation coefficients (r) and levels of significance (p-values) for the studied parameters in the group of patients with RA

Correlated parameters	Type of sample in which mean copper levels/content were assessed		
	Serum [µg/dL]	erythrocytes [µg/dL]	hair [µg/g d.h.m.[1]]
Subject age [years]	r_s=–0.12	r_s=0.06	r=0.28
Mean disease duration [years]	r_s=0.09	r_s=0.17	r_s=–0.03
Ritchie articular coefficient [score]	r_s=0.37**	r_s=–0.02	r_s=0.18
VAS [cm]	r_s=0.24	r_s=–0.15	r_s=–0.17
ESR [mm/h]	r_s=0.49***	r_s=–0.16	r_s=0.06
Hb [g/dL]	r=–0.42****	=0.32*****	r=0.33
DAS28	r_s=0.57******	r_s=–0.15	r_s=–0.10
Cp [g/L]	r_s=0.70******	r_s= 0.23	r_s=0.21

[1]dry hair mass
**p < 0.008
***p<0.0002
****p<0.002
*****p<0.03
******$p<10^{-6}$

a statistically significant, positive correlation was found between the Cp serum concentration and the ESR values (r_s = 0.38; p<0.007).

DISCUSSION

Material for analysis of trace elements' concentration is most often relevant to the extracellular biological compartment, such as the patient's blood serum or plasma [1, 2, 3, 12, 13, 14, 15]. Less often, researchers examine the bioelements' content of the intracellular compartments, such as erythrocytes or hair [4, 10, 16, 17, 18, 19]. The authors of the presented study examined a group of patients with RA, focusing on analysis of concentration/content of environmental bioelements: Copper, Zinc, Magnesium, and Calcium, in both extra- and intracellular compartments. This study particularly focused on the concentration/content of one of these trace elements – Cu – in serum, erythrocytes and hair. The analysis also included the Cp concentration in serum.

The results of the presented study study, related to the serum Cu level in patients with RA, confirmed the reports of some other investigators who have shown higher serum (or plasma) concentration of this bioelement in the group of patients with RA, compared to the control group [1, 2, 3, 4, 12, 13, 15, 16, 17]. According to some other authors, this increased blood serum Cu concentration was even considered to be a marker of clinical activity of this disease [20, 21].

During the inflammatory process, the production of cytokines is increased. Both interleukins: IL-1 and IL-6 are responsible for hepatocytes stimulation to increase the synthesis and secretion of Cp to the blood serum [3, 7]. Ceruroplasmin transfers Cu from hepatocytes to the blood serum (and further, to the tissues) [9]. This may possibly explain the elevated concentration of this bioelement (Cu), as well as the increased Cp level, in serum of our study patients with RA, even though the increased Cp serum concentration in patients with RA has been found only in a few studies [12, 16, 18].

An elevated ESR is considered to be one of the nonspecific biological markers of the inflammatory process in RA [12].

The correlations presented in this study correspond with some data published in the literature related to positive association between the Cu serum concentration and ESR values [12, 16, 18, 21], the Cp serum level [12, 16, 20, 21], and the Ritchie articular index [20]. However, the presented results related to the correlation between Cu levels and ESR values and the Ritchie articular index, are opposite to the findings of some investigators, e.g. Caglayan et al. [3]. This may be caused by a different number of study participants (study sample size) or may be due to the different age structure of the study patients. A substantially higher Cp serum concentration (considered to be an acute phase protein) in patients with RA, significantly correlated with their ESR values, are similar to the results obtained by Louro et al. [12]. The Cu and Cp concentrations increased proportionally to the RA clinical activity. This is considered to be a form of the organism's protective response, since the Cp acts as an antioxidant [5, 12].

No statistically significant correlation between the serum Cu concentration and the duration of the disease (RA) was found in the presented study, which is convergent with the results of some other investigators [3, 16, 18, 21]. Similarly, there was no statistically significant correlation between the serum Cu and the age of study subjects, which was confirmed in the Cu plasma trial by Ala et al. [14]. Therefore, the presented results indicate a need for measuring the serum Cu concentration for evaluation of the RA activity, which has also been suggested by some other authors [16, 21].

The Cu concentration in erythrocytes reveals a higher stability, and is considered to be a better marker of the 'copper status' of the human body than the blood serum Cu level [16]. Only a limited number of studies related to the intracellular Cu concentration, analyzing the erythrocytes of patients with RA, have been conducted [16, 17, 18]. The results of the presented study concur with the results by Tuncer et al., in which the decreased Cu levels in erythrocytes of patients with RA were also revealed [17]. However, the study results of two other investigators quoted above, did not show any significant differences of the Cu concentration in erythrocytes, in relation to the control group [16, 18].

Low levels of Cu in erythrocytes may suggest a lower activity of superoxide dismutase (SOD), which is not performing its function in the cellular anti-oxidative system which, in consequence, may lead to some abnormalities in neutralizing the oxygen free radicals. According to Tapiero et al [7], SOD is one of the first enzymes, which loses its activity in the case of decreased access to Cu. Consequently, the organism's detoxification from oxygen free radicals, which additionally causes the peroxidation of polyunsaturated fatty acids (PUFA) of cell membranes, leading to the production of lipid super-oxides, is decreased. At that time, some proinflammatory mediators and intracellular microelements are released from the damaged cells to the extracellular space [22]. This results in worsening of the inflammatory process. These processes could possibly explain the lower Cu concentration in erythrocytes, although Tuncer et al. [17] have reported that the significantly lower level of Cu in erythrocytes of the patients with RA, compared with the control group, might be due to the increased utilisation of this bioelement in the erythrocytes, as a result of the superoxide dismutase activation.

Hair is considered to be a stable reservoir of microelements [10, 19]. Hair represents a relatively constant compartment in which there are no immediate reactions to some external factors (diet), and thus it remains relatively constant over time, in comparison to some other types of study materials (biological specimen samples) [7, 10, 23, 24, 25]. This is due to the fact that the hair is able to accumulate, during its growth period, within its protein structure, a number of biochemical elements which can illustrate many on-going metabolic processes of the human body [24]. The results of the presented study, in the field of examination of the hair Cu content are substantially different from the findings of Taneja et al, who found the lower Cu content in this compartment among patients with RA [4]. This may be related to some geographical differences between the examined areas, inhabited by various populations, and their relevant dietary habits [26, 27], e.g. some differences in zinc intake which acts antagonistically to Cu [4].

The presented results regarding the Cu levels in the hair of the RA patients are also different from results obtained by Afridi et al. [19]. However, these authors state and give examples that hair Cu levels highly differ between studied populations from various countries and continents [19]. These discrepancies may also be caused by some methodological differences in preparation and examination of the hair, as pointed out by Akyol et al. [28]. According to the presented study data, an assumption can be made that in the group of patients with RA, Cu was shifted from erythrocytes to blood serum and to the hair reservoir.

The study group of patients with RA had been receiving pharmacological treatment for their underlying disease for many years, without making any changes during the last 6 months prior to their enrolment into the study. The role of these medications, in particular diuretics, non-compliance [29] and the impact of different comorbidities, as well as mutual interactions between the above factors and their possible effects on the systemic Cu concentration, are still poorly understood [7, 30, 31]. Based on the presented study data, it is possible to hypothesize that the therapy (presented options 1–4) applied to the study patients' group did not have a significant influence on the Cu concentration in the analyzed compartments. According to the literature, there are only few studies which analyze the effect of the medications used in the RA treatment on the Cu levels in biological compartments examined in this study. Milanino et al. [16] have also revealed that the pharmacotherapy (steroids, NSAIDs, gold, and hydroxychloroquine) used in patients with RA did not cause any significant changes in the Cu concentration, neither in serum nor in erythrocytes. Similarly, Peretz et al [18] found that infusions of methylprednisolone did not modify the Cu concentration, neither in serum nor in erythrocytes. Also, in trial by Onal et al., as in the presented study, no significant influence of MTX, NSAID, GC and SAS was found on serum Cu [1].

CONCLUSION

1. It seems that the 'copper status' in patients with RA, based on the measurement of Cu and Cp levels in blood serum is, correlated with the presence of the inflammatory process.
2. The hair could serve as a useful, additional diagnostic material.
3. Lack of correlation between the Cu concentration/content in serum, erythrocytes and hair, and the MTX and GC dose, may suggest that some other factors that differ

from the applied treatment, can influence the Cu levels in patients with RA.

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