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**PJBS**

ISSN 1028-8880

**Pakistan  
Journal of Biological Sciences**

**ANSI***net*

Asian Network for Scientific Information  
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

## Zinc and Copper Plasma Concentrations in Rheumatoid Arthritis Patients from a Selected Population in Iran

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**Abstract:** The importance of trace elements in chronic inflammatory diseases is related to their cofactor role in immune system functions and in different metabolic processes in articular tissues. The aim of this study was to compare serum levels of Cu, Zn and Zn/Cu ratio in Rheumatoid Arthritis (RA) patients with healthy volunteers in Sari Rheumatology clinic, 2007. Zn and Cu plasma concentrations were assayed using atomic absorption spectrophotometry in 40 selected RA patients sera based on sex and age compared with healthy volunteers. Statistical analysis was performed by SPSS 10 software using independent sample t-test. Zn plasma content in patient group was significantly lower ( $p = 0.02$ ) than that in healthy group. Also, Cu plasma content showed no differences in comparison with healthy group ( $p = 0.15$ ). Results showed no correlation between Cu and Zn plasma concentrations in patient group ( $p = 0.946$ ). In contrast, significant positive correlation was found between Zn and Zn/Cu ratio ( $p = 0.000$ ); but decreased Zn/Cu ratio was more influenced by diminished Zn concentration. The study showed that spreading of RA in Iranian Society is related to age, sex, career and nutrition of the patients. However, consumption of Zn and Cu supplements in RA patients may be suggested by future investigations.

**Key words:** Trace elements, inflammatory diseases, rheumatoid arthritis, articular disorders

### INTRODUCTION

Rheumatoid Arthritis (RA) is an unknown origin chronic disease which is spreading in several systems of human body. Although, the disease shows systemic effects, it includes persistent synovial fluid inflammation like peripheral articular inflammation, which is a major marker of diagnosis of RA related to ability of synovial inflammation to cartilage impairment following articular transformation (Kelly, 2001). Zinc (Zn) is one of the two essential mineral elements which concludes protective mechanisms and treatment of wounds and enhances the antioxidative activity of body; also it has important role in treatment of glaucoma, rheumatoid arthritis, asthma, diabet, hypothyroidism and stress. It can diminish

articular swelling and calcification, based on its antioxidative and synergetic effects on Super Oxide Dismutase (SOD) enzyme (Koda-Kimble *et al.*, 2005). Zn is a vital element which is essential for stability of cellular membrane structure and function and with its antioxidative effect, can protect membrane against unsaturated lipids and inflammatory cytokins (Hennig *et al.*, 1996). Cu deficiency can cause increased sensitivity of lipoproteins to peroxidation and increased DNA oxidative impairment in lymphocytes in culture media (Mazzetti *et al.*, 1996). Several studies showed that decreased content of antioxidative elements, such as Zinc (Zn), Selenium (Se) and Manganese (Mn) and increased content of some elements including Copper (Cu), Cobalt (Co) and Arsenic (As) which probably

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elevate the oxidative stress, can cause cardiac functional disorders (Topuzoglu *et al.*, 2003; De-Lorgeril *et al.*, 2001; Barandier *et al.*, 1999). For instance, two researches showed high serum levels of Cu in ischemic cardiomyopathy (ISCMP) patients (Salehifar *et al.*, 2008); also, the Zn serum levels in idiopathic dilated cardiomyopathy (IDCMP) patients were lower than that in healthy volunteers (Shokrzadeh *et al.*, 2009). On the other hand, effects of Zn and Cu on RA were studied in various investigations that showed decreased Zn serum level and enhanced Cu serum level in RA patients (Grennan *et al.*, 1998; Milanino *et al.*, 1993). Thus, It has been suggested that administration of Zn supplements or Cu chelating agents can be useful for treatment of RA (Brewer, 2005). However, some investigators found normal or higher Zn levels (Hansson *et al.*, 2005) and normal or lower Cu levels in sera collected from control group in their studies. So, they administered Cu supplements to treat their RA patients (Disilvestro *et al.*, 1992; Caldwell, 1999).

This study was performed for determination of mean Cu and Zn serum levels and its relation to RA patients in Sari Rheumatology clinic, Mazandaran Province of Iran.

## MATERIALS AND METHODS

This study was performed as a descriptive-analytical study on 40 RA patients in Sari Rheumatology Clinic in Sari City, Center of Mazandaran Province, 2007.

**Clinical evaluation and selection criteria:** The first group included 40 ISCMP patients and the second group included 40 healthy volunteers with no articular disorder signs.

The young RA patients who were administered orally Zinc sulfate or penicillamine or multivitamin-mineral supplements, were selected as positive control group. In this investigation, the selection criteria for patient and healthy subjects were based on equal age and sex factors.

**Blood sampling and determination of Cu and Zn serum levels:** Ten milliliter blood samples were taken from basilar veins of all patients and healthy volunteers and then were moved in caped experimental tubes, which contained oxalate sodium as anticoagulant; and were heated in water bath (37°C) for 1 h. The samples were centrifuged (1500 rpm) and frozen at -20°C. To determine Zn and Cu concentrations in serum samples, standard Zn and Cu (salt) solutions were prepared. Four standard Zn solutions (0.1, 0.2, 0.3 and 0.4 ppm) and four standard Cu solutions (0.5, 1, 2 and 2.5 ppm) were made. After defreezing, 1 mL of serum samples was collected for assessment of Zn

content and 2.5 mL of serum sample for Cu assessment. The sera were moved to 5 mL volumetric flasks and then glycerol solution 5 and 10% were added for determination of Zn and Cu concentration, respectively. Zn and Cu serum levels were assayed by flame atomic absorption spectrophotometry with  $\lambda_{max}$  = 324.8 nm for Cu level assessment and  $\lambda_{max}$  = 213.9 nm for Zn level determination. Then, the concentrations were determined following evaluation of line equation (Mehmet, 2005).

**Statistical analysis:** Statistical analysis was performed by the SPSS 10 software followed by independent sample t-test to compare the Cu and Zn level of RA patients with healthy subjects and chi-square test for equalization between sex and age factors in control and patient groups. Pearson coefficient was used to study the correlation between Cu and Zn level and age.  $p < 0.05$  was considered to be significant.

## RESULTS

Forty patients in control group were studied that each group included 5 male and 35 female samples. The average of age of the patients was almost 42 years, that the amount of patients in 50-60 year age range was more than that in other age ranges. The groups showed no significant age and sex differences (Table 1).

Comparison between Zn concentrations in control and dose groups showed significant differences ( $p = 0.02$ ), but no significant differences were observed between Cu concentrations in the groups ( $p = 0.15$ ); however, Zn/Cu concentration ratios in the groups had significant differences ( $p = 0.000$ ) (Table 1).

According to determination of correlation of Zn and Cu concentrations and their ratios in the groups, no significant differences was observed between decreased mean Zn concentration and enhanced mean Cu concentration in patients ( $p = 0.946$ ) (Table 2).

The study of serum Zn and Cu concentrations showed that Zn plasma concentration was lower than Cu plasma concentration following increased age in healthy group; and these concentrations were correlated to the Zn and Cu plasma concentrations resulted from the samples which were collected from patients in 40-50 year range.

Table 1: Demographic comparison between Zn and Cu concentrations Zn/Cu ration in dose and control groups

Parameters	Groups		p-value
	Control	Patient	
Sex (female/male)	35/5	35/5	1.00
Age (year)	41.5±14.9	43.7±14	0.90
Zn conc. (ppm)	1.04±0.36	0.71±0.19	0.02
Cu conc. (ppm)	1±0.2	1.08±0.3	0.15
Zn/Cu ratio	1.05±0.36	0.71±0.28	0.00

Table 2: Correlation between Zn and Cu concentrations and with Zn/Cu ratio in RA patients

Serum levels	Zn conc.	Cu conc.	Zn/Cu ratio
Zn conc.	1	-0.008	0.85*
		0.946	0
Cu conc.	0.008	1	-0.488*
	0.946		0
Zn/Cu ratio	0.85*	-0.488*	1
	0	0	

\*p<0.0001

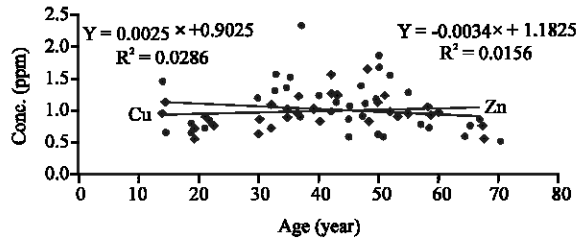


Fig. 1: Zn and Cu concentrations vs. increased age in sample group

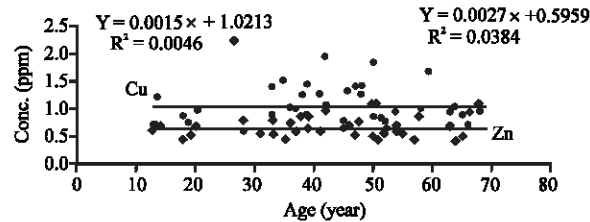


Fig. 2: Zn and Cu concentrations vs. increased age in dose group

However, alteration in these concentrations was in normal range and showed no significant statistical differences ( $p = 0.3$ ) (Fig. 1).

Cu plasma concentration in all age ranges in dose group (RA patients) was significantly higher than Zn plasma concentration ( $p = 0.000$ ) and the both plasma concentrations was increased by parallel following age enhancement (Fig. 2).

### DISCUSSION

The role of Zn was established in growth, haematopoiesis, wound treatment rate and production of nucleoproteins; also, this vital element is an essential moiety of more than 1200 enzymes in human body (Mazzetti *et al.*, 1996). Zn can subside articular swelling and morning stiffness (Koda-Kimble *et al.*, 2005; Mazzetti *et al.*, 1996). Otherwise, Cu has an important role in function of many enzymes and its deficiency can cause dysfunction of peroxidase and catalase enzymes (Mazzetti *et al.*, 1996).

This study showed that the number of female RA patients is more than male ones (88% female: 12 % male). Increased number of female patients can be related to more possibility of autoimmune diseases in this genus; and perhaps, specific female hormones are one of the predisposing factors of this disease which spreads in 0.8% of population (0.3-2.1%) (Kelly, 2001). On the other hand, occupational study showed that most of female patients were as housewives and their educational level was up to diploma. In this study, Zn plasma concentration in the patients was lower than that in healthy group ( $p = 0.02$ ), that the results was same as the consequences resulted from other investigations (Grennan *et al.*, 1998; Milanino *et al.*, 1993). Zoli *et al.* (1998) showed that the Zn serum level in 57 female RA patients was significantly lower than that in sample group; also, they manifested an inversed relationship between Zn serum levels and inflammatory factors. Calcareous soil, lack of Zn absorption in wheat and grains and its deficiency in bread as an important daily food regimen are some factors which can effect on this relationship.

This investigation showed no significant differences between serum Cu levels in control and patient groups ( $p = 0.15$ ), but other researchers manifested high Cu plasma concentration in RA patients and its relation to the disease (Brewer, 2005; Amancio *et al.*, 2003). These results may be related to the nutrition of people who live in different geographical areas with various nutritional habits; and serum Cu and Zn levels could be affected by trace element content of food. Based on Table 2, there was no correlation between Zn and Cu concentrations ( $p = 0.946$ ); thus, following increase of Cu content, Zn content decreased in the patients. However, significant correlation was observed between Zn and Cu concentrations ( $p = 0.0001$ ). No relationship was found between concentrations of these elements and intensity of RA, but the Zn/Cu ratio had a relationship with the disease (Caldwell, 1999).

As indicated in Fig. 1, Zn plasma concentration was lower than Cu plasma concentration following enhancement of age of patients. However, alteration in Zn and Cu concentrations was in normal range and showed no significant differences ( $p = 0.3$ ), but Cu plasma concentrations were significantly higher than Zn plasma concentrations in all age ranges in RA patients ( $p = 0.00$ ), (Fig. 2). The higher age (50-60 years) was led to higher plasma concentration of both elements.

Ultimately, based on the results of this study and diminished Zn level in RA patients, Zn supplements were suggested as complementary diet to treat RA patients.

Also, serum Cu levels in RA patients were insignificantly higher than health volunteers; so, major notification is necessary for improvement of Zn supplementation in danger-faced groups of society who have risk factors of RA. On the other hand, this investigation must be performed with equipped studies as clinical trails in different societies to organize the nutritional programs of governmental health organizations.

#### ACKNOWLEDGMENT

This study was supported by a Grant from the Research Council of the Mazandaran University of Medical Sciences, Iran.

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