SERUM COPPER AND ZINC LEVELS AND COPPER/ZINC RATIO IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Abstract

Background: Trace elements particularly copper and zinc have a great role in a number of biological processes, therefore estimation of these metals in serum of Rheumatoid Arthritis (RA) patients may ameliorate the understanding of trends in the relationship between the serum copper and zinc levels and activity of disease.

Objective: To determine the serum copper and zinc levels and its relation with the degree of disease activity and compare with a normal group.

Methods: The investigation was carried out on forty-three healthy (25 females, 18 males) and fifty-four(37 females, 17 males) sick adults.The patients were subdivided according to the activity of the disease(Tentative EULAR Criteria) in to two groups. Of them 29 patients (21 female, 8 male) had low disease activity [age range 32-65 years] and twenty-five patients(16 female, 9 male) had high disease activity [age range 22-52 years]. They were compared with 43 healthy control [age range19-64 years].
Serum copper and zinc were determined by using the atomic absorption specrtophotometry.

Results: Serum copper level and copper/zinc ratio in patients with RA in both groups were significantly
increased than the control group. In addition the serum zinc level in patients with low activity RA was significantly decreased and more in high activity disease.

Conclutions: As a result, the alteration of copper and zinc levels in the sera patients with RA can related to degree of disease activity and enable to shed more light on the role of trace elements in both physiological and pathological states.

Key words: Rheumatoid Arthtitis, copper, zinc, and serum.

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Introduction

Metal ions have a great variety of functions in biochemistry. They serve as catalyst in active center of enzymes, as factors stabilizing the structure of the enzymes or as a counter ion to provide electro neutrality. Metal ions in human serum have various functions. They act as the physiologically active part of enzymes (copper in coeruloplasmin) or they are bound to proteins in order to be stored (iron in ferritin) or transported (iron in transferrin)[1].

The trace elements copper and zinc have a significant role in antioxidant protection and immunity. They are constituents in antioxidative enzymes, zinc and copper in cytoplasmic superoxide dismutase[2] and copper in ceruloplasmin[3], which is an important antioxidant in serum[4]. Zinc is important in the maintenance of proper immune response[5,6]. In inflammatory conditions, such as rheumatoid arthritis (RA), there are alterations in blood and serum concentrations of these micronutrients.

Serum zinc was found to be reduced and serum copper increased in adult rheumatoid arthritis and these variations appeared to be associated with the immune inflammatory rheumatoid

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process[7]. Neidermeier and Griggs[8] reported low serum zinc values in-patient with RA. Similar observations have been reported by other researchers[9,10] Moreover, both in humans[11] and in animals[12] zinc preparations have been shown to be anti-inflammatory and antiarthritic agents. In contrast, Alegree, et al[13] reported normal serum zinc values in-patient with RA. Low serum zinc and zinc deficiency in patients with RA could be the results of zinc deficient diet, therapeutic regimens, shift of zinc from plasma to other body compartements, and/or increased urinary zinc excretion.

Our purpose was to elucidate the alteration of copper and zinc concentration in sera patient with RA and to evaluate the relation between the levels of copper and zinc with various degrees of disease activity compared with a normal control group.

Materials and Methods

Chemicals: Standard solution for copper and zinc of 1000µg/ml concentration (Atomic absorption grade) were obtained from Aldrich chemical company LTD. All solutions were prepared from analytical grade chemical and distilled deionized water was used throughout.

Apparatus: Serum copper and zinc were measured with a shimadzu- flame atomic absorption model AA-646. Single elements hallow cathode lamps were used as line-radiation sources for individual elements and were operated at current recommended by the manufacturer and the manual of the atomic absorption spectrometer.

Preparation of standard working solutions: On the day of determination, the working solutions were prerared by sequential dilution (i.e.standard 1000µg/ml was diluted to 10µg/ml from which the lower concentration of working solution were prepared sequentially) to obtain the following concentration of the working standard (0.4,0.8,1.2,1.6 and 2.0µg/ml) of copper and zinc.

Frozen sample were allowed to thaw and come to room temperature then mixed gently. Sample were diluted 1:10 with (6%) butanol as diluents, this method achieved 30% increases in sensitivity compared to use of water only[14].

Patient's selection and sample collection:

Subjects: The clinical part of this study was completed between April and August 2004 in the Rheumatology Outpatient Clinic in Baghdad Teaching Hospital-Medicine City. The study population consisted of ninety-seven healthy and sick adults. Fifty-four (37 female, 17 male) from the Rheumatology Out patients' clinic were diagnosed as having RA according to the Rheumatoid Arthritis Disease Activity Index (RADAI) for epidemiological research[15].

Forty-three healthy persons made up the control group (Group1). Age range of the control group was 19 to 64 (mean 44.3±4.2); male/female ratio was 0.72. The patients with RA were divided into 2 groups after evaluation of their disease activity by the Tentative EULAR Criteria[16]. [Number of tender joints: maximum 28; number of swollen joints; maximum 28; pain score:0 (no pain) to 10 (very sever pain), erythrocyte sedimentation and morning stiffness of at least 30 min duration].

Twenty-nine patients (21 female, 8 male) with less than 14 swollen joints and one hour morning stiffness were considered to have low activity of the disease; these patients made up Group 2 and 3. Another twenty-five patients (16 female, 9 male) presenting a higher number of swollen joints and more prolonged morning stiffness, were considered to have a high degree of inflammation and were defined as Group 4 and 5. Characteristics of the

groups 2, 3, 4 and 5 are presented in tables 1 and 2.

Table 1: Characteristics of the Rheumatoid Arthritis Groups (2) and (3) low activity

Data	Group 2	Group 3
Sex	Male	Female
No. of patients	8.0	21.0
Age range (years)	32-65	19-16
Age (years)	55.30±1.90	48.8±2.30
Duration of disease (years)	6.90±1.10	5.90±1.20
Morning stiffness (hours)	0.70±0.20	1.10±0.30
No. of tender joints	15.20±3.0	16.20±2.30
No. of swollen joints	6.10±1.20	8.90±2.0
ESR (mm/h)	36.0±8.0	34.0±2.0
+ve rheumatoid factor	5/8	17/21

Table 2: Characteristics of the Rheumatoid Arthritis Groups (4) and (5) high activity

Data	Group (4)	Group (5)
Sex	Male	Female
No. Of patients	9.0	16.0
Age range (years)	22-52	19-58
Mean age (years)	45.6±7.9	43.8±4.8
Duration of disease (years)	5.2±1.2	6.6±2.3
Morning stiffness (hours)	3.4±1.1	3.3±0.3
No. Of tender joints	18.9±2.3	24.0±0.8
No. Of swollen joints	15.1±3.5	14.2±2.5
ESR (mm/h)	65.0±5.0	78.0±5.0
+ve rheumatoid factor	9/9	16/16

Serum preparation:

Avenues blood samples were drown by utilizing disposable needle and plastic syringes from each patient and control. The blood was allowed at room temperature for 10 minutes for clothing; centrifuged at 3000 rpm for 10 min, then serum was separated and stored at (-20^{o}C) until analysis.

Accuracy and Precision:

The accuracy and precision of the method were checked. Table 3, shows the relative standard deviation and relative error percent for five replicate results obtained for lowest and highest concentration range of each individual constituent.

Table 3:Accuracy and precision of copper and zinc analysis.

Cu added µmol/l	Cu found µmol/l	Relative Standard Deviation %	Relative Error
0.0	9.6	-	-
1.40	11.31,11.21 11.12,11.01 11.11.	1.0	1.36
3.50	13.10,13.12, 13.12,13.15 13.18	0.24	0.24
5.65	15.20,15.25 15.31,15.15 15.12	0.50	0.32
Zn added µmol/l	Zn found µmol/l	Relative Standard Deviation %	Relative Error
0.0	5.44	-	-
2.50	7.85,7.92 7.88,7.90 7.95	0.61	0.47
5.0	10.60,10.51 10.31,10.45 10.51	1.01	0.34
7.20	12.52,12.62, 12.56,12.55 12.60	0.27	0.58

Results and discussion

The current study provides data on copper and zinc levels in Rheumatoid Arthritis sera patients and compared with those of normal subjects. As different degree of inflammation may specifically possess different characteristics of the disease and different levels of these elements, separate calculation were made for each test.

Normal subjects:

Serum total copper and zinc were detected in normal subjects. The normal values for copper and zinc in female

was (18.60±1.24) and (15.07±0.66) µmol/l while they were (15.87±2.1) and (14.07±0.85) µmol/l in male respectively, the results show little differences between females and males for both copper and zinc, so that the total means for both sexes was (17.23±0.91) and (14.57±0.21) µmol/l respectively, as show in table 4.

Table 4: The serum copper, zinc levels of the patients with RA and control

Cases	Serum Cu	
	Mean ± SD µmol/l	Mean ± SD µmo l/l
RA with low activity		
Female (21)	21.39±3.31*	8.36±0.79*
Male (8)	19.69±2.31*	8.07±1.03*
RA with High activity		
Female (16)	32.25±6.12*	5.33±0.92*
Male (9)	29.40±4.46*	4.62±0.98*
Total (54)	25.68±1.22*	6.66±1.23*
Normal		
Female (25)	18.60±1.40	15.07±0.66
Male (18)	15.87±2.0	14.57±0.85
Total (43)	17.23±0.1	14.57±0.21

* = P <0.001

Rheumatoid Arthritis (AR) patients:

The results of sera samples from patients suffered from (RA) compared to these of normal subjects, were generally characterized by increase and decrease levels of copper and zinc respectively.

Serum copper level for patients and normal are shown in table 4. In general the copper concentration in 54 (Fifty-four) patients sera samples was found to be 25.68±1.22 µmol/l, while the corresponding level in 43 (Forty-three) healthy subject was 17.23±1.80 µmol/l. This increase of 49% in copper level was statistically significant (P< 0.001).

As different degree of inflammation may specifically possess different activity of the disease lead to different concentration of copper, separate calculation were made for each characteristics of the disease test. Both in the normal donors and in the various groups of patient, these were significant difference between values obtained for male and female; therefore they were grouped independent. The mean serum copper levels were found to be 21.39±3.11µmol/l and 19.69±2.31 µmol/l for female and male respectively with low activity patient; and 32.25±6.12 µmol/l, 29.40±4.46 µmol/l for serum female and male with active disease.

The magnitude of the increase in the value varied between 24% for female and 14% for male patients with low activity. In contrast, the magnitude of the increase in serum copper level varied between 87% for female and 70% for male patient with active disease.

Figure 1 shows a histogram of the distribution of the serum copper levels in the normal donors and in the (RA) patients grouped according to the activation and severity of disease.

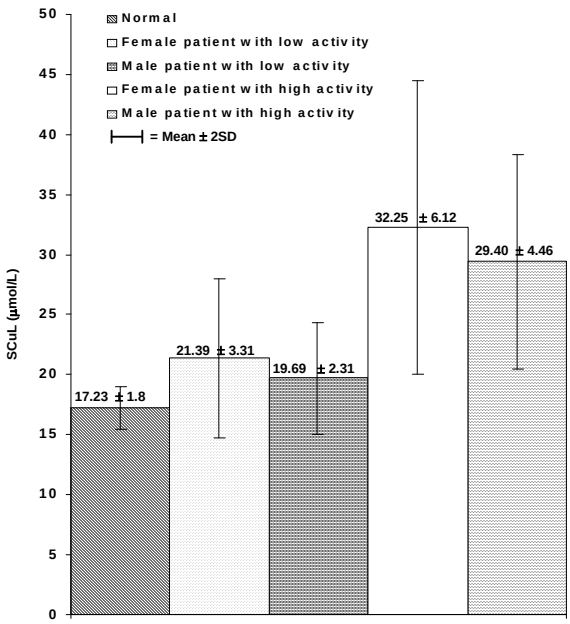

Figure 1: Serum copper level in normal and in patients with RA

The mean level of the serum copper in the total groups of (RA) patients was significantly elevated compared to the levels of the normal donors; nevertheless, only 4 of 21 (20%) and 4

of 8 (50%) for female and male patients with low activity disease have serum copper levels higher than 2SD (standard deviation) above the mean for normal donors. Of these all cases of proven active (RA) had copper serum levels higher 2SD than mean for normal.

The most pronounced changes were found in the levels of serum zinc (Table 4). The mean zinc level in 54 normal was found to be 14.57±0.21 µmol/l, while the mean zinc in all the patients with (RA) was 6.66±1.23 µmol/l, this 56% decrease in the zinc level was statistically significant (P<0.001). The results show no significant differences between values obtained for female and male. The decrease in the zinc serum level was more pronounced when the patients were grouped according to the high activity of the disease, the magnitude of the decease in the value varied between 63% for female and 68% for male with active disease.

Table 5 shows the results obtained for serum copper, serum zinc levels and cu/zn ratios of the patients with RA and the controls.

Table 5: The mean serum copper, serum zinc levels and copper/zinc ratios of the patients and normal.

Cases	Serum Cu µmol/l	Serum Zn µmol/l	Cu/Zn µmol/l
RA with low activity			
female (21)	21.39±3.31	8.63±0.79	2.47±0.52
male (8)	19.69±2.31	8.07±1.01	2.43±0.24
RA with high Activity			
female (16)	32.25±6.12	5.33±0.92	6.05±0.66
male (9)	29.40±4.46	4.62±0.98	6.36±0.41
Total (54)	25.68±1.22	6.66±1.23	3.93±0.43
Normal (43)	17.23±1.80	14.57±0.21	1.17±0.29

It was found a highly significant differences (P <0.001) between the copper/zinc ratio for the control group and that for the patients group, this differences is the result of both higher values for copper and lower values for zinc in serum from the patients group as compared with normal persons. In all cases of proven active disease, the cu/zn ratio was significantly higher as compared to low activity patients group.

Copper and zinc are very effective in DNA and RNA synthesis and cell division. These elements are required for the activation of enzymes such as timidin Kinase, RNA-polymerase and DNA-polymerase.Zinc also play an important role in the catabolism of RNA by regulating RNase activity.These two metals prevent the formation of free radicals capable of mutation with the antioxidant effect[6,17,18].

One approach to the assessment the role of copper and zinc in inflammatory conditions, such as (RA), analogous to that used for other nutrients, is measurement of copper and zinc in the serum or plasma over time because they are co-factors of important enzymes involved in collagen and bone metabolism[19] and immune system function[20]. Results studies have demonstrated that both copper and zinc alterations can be explained by the active inflammatory process and that serum trace elements are measures of disease activity.

Our studies show elevated level of copper in serum of (RA) groups and decrease level of zinc in serum patients with low activity and a more decreased in high (RA) disease activity. Change in serum zinc and copper levels observed in RA patients led some investigators to hypothesis that a marginal deficiency in zinc and copper might contribute to the development of RA and to the progression of the disease itself.

One of hypothesis of decrease zinc and increase of copper in sera of acute or chronic inflammatory processes cause an accumulation of copper and zinc in many body compartments and in the inflamed ares, supporting the hypothesis that the development of inflammation induces an increased body requirement of copper and zinc

containing proteins and enzymes such as metallothioneins, ceruloplasmin and Cu-Zn-superoxide dismutase[7,18,21]. Our results in both RA groups confirm those of studies that observed low plasma or serum zinc in patients with RA[22,23,24], and the results also show that serum copper and zinc levels are correlate to severity of disease.

Low serum and plasma zinc could be regarded as a secondary phenomenon arising from the hypoalbuminemia observed in patients with RA[25]. Levels of both zinc and albumin were found to be decreased by about 12% in plasma and correlated significantly with each other in patients with RA[7].

However, it seems that the decrease in serum zinc is independent of the reduction in circulating albumin[7]. This hypothesis is supported by data showing that only 1.10-2.6% serum albumin in humans' functions in zinc transport and that zinc occupies less than 0.2% of the total zinc binding capacity of the protein[26]. Other investigators attribute zinc deficiency in patients with RA to inadequate nutrition[27].

One of the hypothesis proposed to explain the decreased in plasma or serum zinc is due to redistribution of zinc in the organism, including in particular accumulation of the metal in the liver[28]. In contrast, Alegre et al[13] reported normal serum zinc values in patients with RA, the weakness of that study was the lack of measurement of serum levels in a control group.

Michaelsson and Ljunghall[29] reported decreased zinc concentrations in the serum and epidermis of men with dermatitis herpetiformis, which is considered an autoimmune disease. Svenson et al[10,30] examined zinc concentration in peripheral blood cells; erythrocytes, granulocytes and platelets. Therefore, at this stage of research, it seems that zinc malabsorption is not a specific defect of RA, but rather is common to other inflammatory connective tissue diseases.

Since zinc may act as a stabilizer of various biological membranes by interacting with the extrinsic macromolecule components of the membrane mainly the enzymes and/or directly with the intrinsic structure of the plasma membrane, therefore the low zinc level will affect the membrane stability as well[31].

Zinc deficiency results in blunted cellular immunity, measured by in vitro lymphocyte response to mitogens and delayed cutaneous hypersensitivity testing[32]. In addition, neutrophil chemotoxis is slowed; natural killer cell function is reduced[33,34]. It is possible that zinc deficiency may perpetuate disease in patients with RA through immunocompromised cellular mechanisms. This hypothesis calls for a prospective double blind study of the effect of zinc adminstration on clinical manifestations and cellular immunological functions of patients with RA.

The model involvement of increase in serum copper particulary serum cupro-enzyme and ceruloplasmin which was observed in various specific and non specific pathological condition and was attributed in biological damage caused by superoxide, a radical found in all living tissues, according to this model, superoxide radical or other reducing agents, such as ascorbate, reduced the copper complexes to the cuprous state. In turn, these complexes react with hydrogen peroxidase to form hydroxyl radicals that damage proteins, RNA and most important DNA.Repetitive formation of OH radicals at a specific location-where the copper ions are found is probably the mechanism of this process. These radicals may cause double strand break in the cellular DNA that are not repairable by cellular mechanisms[35].

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