SERUM ZINC AND COPPER LEVELS IN RHEUMATOID ARTHRITIS

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SUMMARY: Rheumatoid Arthritis, a chronic multi-system disease, causes many systemic manifestations, the most characteristic of which is the symmetrical involvement of peripheral articulations by inflammatory synovitis. There is also a consideration that cellular immunity has some role in the genesis of the disease. Indeed, RA has been described as "T-Iymphocyte- macrophage immunoregulation disorder".

There is also a considerable amount of evidence indicating that Cu and Zn may contribute in the etiopathogenesis of the disease. Some earlier studies indicated that metabolism of Cu and Zn is substantially altered in patients with RA, suggesting a potential role for Cu and Zn in the development of the disease.

In the present study we observed significantly higher serum Cu levels in patients with RA compared with those in normal control subjects ($P<0.0001$). Cu levels in active patients were somewhat higher compared to non active RA patients ($P<0.005$).

On the contrary the results for serum Zn levels patients with active RA had lower serum Zn levels when compared with normal control subjects ($P<0.001$). This difference was also statistically important between active and non-active patients ($P<0.05$).

Key Words: Rheumatoid Arthritis, Zinc, Copper.

INTRODUCTION

The etiology of Rheumatoid Arthritis (RA) still remains an enigma. RA is a chronic disease that causes systemic and articular inflammation, and is seen in all races and ethnic groups around the world (1-3). Most commonly mentioned culprits are: heredity, infectious agents, and sexual factors (1).

Zn is a crucial element in a series of cellular functions as normal growth, protein metabolism, membrane stability, and metalloenzyme functions (4-6). Zn, has several other effects on immune response, complement system, lysozomal enzyme release, and macrophage functions (5,7). Zn is also indispensible in many steps of the inflammatory reactions. Among these are prostaglandin biosynthesis, stimulation of lymphocytes and immune response, and scavenging of toxic free oxygen radicals (8-10). Zn is likewise an important element in collagen tissue formation and bone metabolism (7,9).

There is an abundance of Cu in the human body and nature (11,12). Cu is incorporated into the structure of a great many enzymes and proteins (12-14).

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RA, as a chronic inflammatory disorder, can cause substantial elemental alterations in the body (15). Inflammation induces consumption of Zn and Cu (15). RA, during the course of the disease, cause elevations in some plasma cytokins, as IL-1, IL-6, and TNF (4,15,16). These cytokins induce the synthesis of metallo-enzymes and thus, sequestration of Zn in the liver, pancreas and intestine. This results in a decrease in serum Zn level (4,9,15,17-19). Besides these, IL-1, and IL-6 initiate the synthesis of a series of acute phase reactants among which ceruloplasmin is involved and cause alterations in metabolism of Cu and Zn (20,21). It is considered that decreased Zn level, in turn, results in T- lymphocyte function disorder, a possible factor in RA pathogenesis (7).

Cu tiofan, a complex that prevents local tissue damage, is detected within normal limits in healthy individuals and however, is found lower in patients with RA (22).

Our aim in this study was to investigate whether a potential role could be assigned concerning the ethiopathogenesis of these elements (Cu and Zn) by means of comparing serum level of these elements in patients with RA with those in normal subjects.

Additionally we sought for a possible correlation between the clinical and laboratory activity of the disease and the serum levels of these elements.

MATERIALS AND METHODS

The study consisted of a total of 40 patients (32 females and 8 males) who received the diagnosis of RA according to the criteria outlined by ARA. We constituted a control group of healthy individuals (6 males, 14 females) picked out from the hospital staff. All the patients were examined meticulously at the onset of the study. Additionally a thorough articular examination was performed for each patient to assess any articular tenderness and swelling by means of Ritchie Articular Index (23-24). Finally functional capacity for each subject was determined and noted regarding the classifying criteria of Steinbrocker (25). A-P and lateral X-ray roentgenograms of the hands of each patient were obtained to evaluate 20 articulations in the hand (10 Metacarpophalangeal, 8 proximal interphalangeal, 2 interphalangeal junction of the thumb) (26).

The term "active disease" meant that the patient had a minimum of three articulations involved, at least 9 sites of painful tenderness on digital compression, and had a morning rigidity exceeding 45 minutes and an ESR over 28 mm/hour (27). On the other hand the term "disease in remission" required that the patient had no articular involvement and symptoms, and had a morning stiffness below 15 minutes and ESR less than 20 mm/hour.

According to the foregoing definitions, 29 patients had active disease and 11 were under remission, haemograms were obtained for each individual patient. ESR estimation was performed by Westergren technique in each patient. ASO, CRP and RF levels were evaluated by standard biochemical techniques. A series of biochemical analyses were performed for each subject consisting of BUN, creatinin, Na, K, Alkalen Phosphatase, Ca, P, SGOT and SGPT. Patient revealing abnormal results of anyone of these tests was discarded from the study. Having completed the detailed studies, we constituted the study group consisting 40 RA-patients and 20 normal healthy patients. We collected serum specimens from the two groups at different times. Infections with 10 cc-injectors were done for all the patients who were fasting from the night before. Blood Specimens were santifuged at 5000 rpm for fifteen minutes. Serum specimens obtained by santrifuge and were stored at (-20°C) until the time of analysis. Serum Cu and Zn levels were estimated by Perkin-Elmer 103 atomic absorption spectrophotometry. Results were documented in terms of mcg/dl. The results of the analysis were confirmed by Mann Whitney U test and Pearson correlation analysis. $P<0.05$ results was regarded positive.

RESULTS

Our study was conducted in Ankara Numune Hospital at the Physical Therapy and Rehabilitation Clinic. The study involved 40 patients with RA and 20 normal subjects. Ages of the patients varied from 19 to 70 with a mean of 49.9 ± 10.3 while in the control group varied from 32 to 60 years with a mean of 49.2 ± 7.9 years. There was no significant difference between the two groups with regard to age ($P>0.05$).

Duration of the disease varied from 6 to 240 months in RA group with a mean of 68.4 ± 52.1 months. Duration of morning stiffness varied from 15 to 240 minutes with a mean of 90 ± 50 minutes. Radiological damage score of some patients was between 15 and 83 with a mean of 28.9 ± 12.4. Erosional score was between 1

and 19 (median 7.1 ± 3.4).

40 RA patients were divided into two groups, active and inactive RA groups.

Laboratory data analysis from RA patients demonstrated that the changes in Hb was between 8.5 and 15.8 gr (12.07 ± 1.7), leukocyte count was between 4100 and 12500/mm^3 (7210 ± 2100/mm^3), platelets count was between 184900 and 490000/mm^3 (mean 348.980 ± 80.090 mm^3), ESR was 25-127 mm/hour (mean 59.6 ± 26.6 mm/h), serum CRP was 6-128 IU (mean 41.2 ± 39.7 IU), RF was positive in 27 patients with RA (67.5%), RF was positive in 5 patients with inactive RA (45.5%), RF levels were 70.9 ± 58.9 IU in patients with inactive RA, while it was 91.2 ± 88.9 IU in patients with active RA. There was no significant difference between two groups (P>0.05).

Mean total protein levels 72.3 ± 4.3 mg/dL in patients with inactive RA. Mean total protein levels 81.7 ± 59.1 mg/dL in patients with active RA. There was no significant difference between two groups (P>0.05). Albumin levels were 44.3 ± 3.8 mg/dL in patients with inactive RA, and 47.2 ± 11.1 mg/dL in patients with active RA. There was no significant difference between two groups (P>0.05).

Serum Cu levels were 139.4 ± 25.4 mg in patients with RA, and 98.2 ± 11.5 mg in healthy control groups. The difference was significant between two groups (P<0.0001). Serum Cu levels were 131.8 ± 22.3 mg in patients with inactive RA, and 142.2 ± 26.2 mg in patients with active RA. But there was no significant difference between two groups (P>0.05).

Serum Zn levels were 71.8 ± 11.9 mg in patients with RA, and 80.9 ± 7.8 mg in healthy control group. The difference was significant between two groups (P<0.001). Serum Zn levels were 77.5 ± 9.1 mg in patients with inactive RA, and 69.7 ± 12.2 mg in patients with active RA. There was significant difference between two groups (P<0.05).

Correlation between morning stiffness in patients and ESR were positive (r:0.524, P<0.001). A positive correlation was also observed between morning stiffness in patients and serum CRP (r:0.358, P<0.05).

Correlation between morning stiffness and serum alkaline phosphatase levels was likewise positive (r:0.358, P < 0.05).

Correlation between ESR and serum alkaline phosphatase was positive (r: 0.425, P < 0.01).

Positive correlations between Ritchie Articular Index and ESR and also RF were observed (r: 0.489, P<0.001 and r: 0.333, P<0.01).

Positive correlation between ESR and RF in patients with RA was observed (r: 0.419, P < 0.01).

Negative correlation between serum Zn levels and disease duration was noted (r: 0.248, P < 0.05).

Negative correlations between serum Cu and serum Zn levels and serum Alkaline phosphatase were observed (r: 0.402, P < 0.01 and r: 0.328, P < 0.05).

DISCUSSION AND CONCLUSION

Diseases ultimately resulting in chronic arthritis, as in the case of Rheumatoid Arthritis, give rise to some sequela and discomfort to patients despite long lasting treatment, causing a great economic burden (28). A recent report from Finland indicates a striking decline in the prevalence of RA in the last several decades. The report documents the results of two prospective studies, in a follow-up period of 9 years starting from 1959, (38%) patients became confined to bed. In the other study, follow-up period of the same duration starting from 1982, 2% became confined to bed. Factors contributing to this decreasing trend are several: early recognition of the disease due to improved clinical and serological diagnostic techniques, increased diagnostic rate in mild cases, early provision of second step treatment (29). RA, as is noted above causes diminished man power, physicosocial predicaments, and restricted daily activity in patients. Thus, patients with the disease should promptly be diagnosed and given the appropriate treatment.

In this study we found a spectacular relationship between factors determining the activity of the disease (ESR, CRP, Alkaline Phosphatase). Previous studies maintained a heightened Alkaline Phosphates level in patients with active RA due to increased bone metabo-

lism in these patients (30-33). In this study such correlation is an evidence that bone destruction is a continuous process in active disease processes.

Inflammation within tissues induces a series of anti-inflammatory responses in which a number of proteins and enzymes carrying Zn and Cu elements are involved. Most notable among these are; metallothioneins, ceruloplasmin, and SOD (16,34).

Intracytoplasmic SOD includes both Cu and Zn, ceruloplasmin, a powerful antioxidant in serum carries only Cu (8). Substantial alterations in metabolisms of Cu and Zn occur through some physio-logical control mechanisms over an inflammatory reaction (15). Indeed, inadequate serum levels of Cu and Zn are associated with a defect in antioxidant mechanism and thus, are considered potential culprits in the genesis of the disease (16). Plasma Zn concentration is determined by many factors; nutritional status and history of previous infections of the patient and others (16.35). Also some studies demonstrating decreased plasma Zn levels in RA patients compared with normal subjects, lend support for the idea that reduction of the Zn element is essential in the genesis of the disease (5,16,17).

In this study, we observed decreased Zn levels in patients with RA with respect to normal individuals. We also found out that active RA patients had more substantial decreases in serum Zn levels compared with inactive patients. This statistically important difference suggests the critical role of inflammation in serum Zn depletion. There is considerable evidence from previous studies that, Zn distribution between the body compartments is reorganized by inflammatory process. Through this distribution, serum Zn level falls while Zn in mononuclear leukocytes, synovial liquid, and urine level rises (36). Continuous hepatic synthesis of acute phase reactants during an inflammation is held responsible for this pathologic distribution (4). Factors mediating the synthesis of acute phase reactants are ACTH, Cortizol and Cytokins (IL-1, IL-6, TNF). These mediators induce the synthesis of metallothioenins and overburdening of Zn in the liver (4,9,11). This, inturn, results in serum Zn depletion in patients with active RA. Data from previous studies suggested a correlation between the extent of inflammation and serum Zn depletion (15).

Another observation in this study is the existence of a correlation between serum Zn depletion and the laboratory and clinical indexes of active RA (Ritchie Articular Index, ESR, CRP).

This finding shows that serum Zn decreases result from such several factors as trauma, infections, and medical treatment of the disease (16).

The meaningful correlation between serum Zn levels and the duration of the disease, as also is seen in the present study, may be in part, secondary to a nutritional disorder encountered in chronic inflammatory diseases (37). Further confirmation of this idea requires repetitive screening of serum Zn levels in the same patient group on a prospective basis.

Anti-inflammatory and anti-arthritic properties of Cu are demonstrated on both animal and human studies (15). Cu concentration is two fold in an inflammatory region, serum Cu elevations, once attributed anti-inflammatory phase to the inflammatory phase reactions, are now considered dependent on acute phase reactants (38,39). It is reported that 30 to 50% increases in serum Cu level during an acute phase response triggered by IL-I release largely depend upon the increased synthesis of ceruloplasmin.

It is also demonstrated that ceruloplasmin increases during acute phase reactions in order to scavenge toxic free oxygen radicals (15,20,40).

Our study showing that patients with RA have markedly elevated serum Cu levels compared with normal subjects, lend support to the reports indicating altered serum Cu levels in collagen tissue diseases.

On comparison of active patients with non-active patients with respect to serum Cu levels, we found no statistically important difference but active group seemed to have higher levels. In two different studies performed in 1982 and 1983, direct correlation was observed between the activity of the disease and serum Cu levels. However, recent reports do not con-

firm such a correlation (16). As we noted in our study no correlation exists between active and inactive patients in terms of serum Cu levels. We found no meaningful relationship between serum Cu levels and clinical and laboratory activity of the disease (ESR, CRP, Ritchie Articular Index). We therefore conclude that some other factors than activity of the disease are responsible for the alterations in serum Cu level in RA.

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