

SIGNIFICANT CHANGES IN SERUM COPPER AND ZINC LEVELS IN PATIENTS WITH RHEUMATOID ARTHRITIS.

التغيرات المعنوية في مستويات الزنك والنحاس في أمصال المرضى المصابين بالتهاب المفاصل الرثوي

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Abstract:

objective of this study was to measure the alterations in two of important serum trace elements, including zinc (Zn^{++}) and copper (Cu^{++}), in patients with rheumatoid arthritis (RA) in AL-Hakeem hospital in Najaf city. In the study, serum trace elements were determined by using Atomic Absorption Spectrophotometer in sera of patients with RA (n=22), and statistically compared with healthy individuals (n=12). Serum Cu^{++} concentration was found significantly higher in patients group than those of healthy individuals (29.06 ± 3.78 and $20.74 \pm 4.30 \mu\text{mol/L}$, respectively) ($p < 0.01$). In contrary, Zn^{++} levels were lower in patients ($7.78 \pm 4.41 \mu\text{mol/L}$ and $13.55 \pm 6.00 \mu\text{mol/L}$, respectively) according to the healthy subjects ($p < 0.05$).

The present study indicate no significant changes between active RA group and non-active group patients copper when evaluated copper metal. But there was significant difference between two groups ($p < 0.05$) for zinc metal. Additionally positive correlation between serum cu levels and disease duration, while the correlation between serum Zn levels and disease duration was negative.

Our results showed a considerable amount of evidence indicating that cu^{++} and zn^{++} may contribute in etiopathogeesis of RA disease and that serum (Zn^{++}, Cu^{++}) concentrations are altered in RA patients ,we suggest that potential role for Cu and Zn may cooperate in the development of the disease .

// الخلاصة //

الهدف من هذه الدراسة هو قياس التغيرات التي تحدث في تركيز اثنين من أهم العناصر ألا وهما النحاس والزنك عند المرضى المصابين بالتهاب المفاصل الرثوي في مستشفى الحكيم في محافظة النجف الاشرف .تم تقدير هذه العناصر في أمصال دم ٢٢ مريض و ١٢ من الأفراد الأصحاء باستعمال جهاز قياس الامتصاص الذري .

لوحظ زيادة معنوية ($p < 0.01$) في أمصال دم المرضى عند مقارنتها مع الأصحاء عند تقدير عنصر النحاس . أما عند تقدير عنصر الزنك فوجد انخفاض معنوي ($p < 0.05$) في أمصال دم المرضى عند مقارنتها مع الأصحاء بالاطافة إلى ذلك لوحظ انه ليس هناك فروق معنوية في تركيز النحاس في كلا مجموعتي المرض الفعالة وغير الفعالة ($p > 0.05$) . بينما هناك انخفاض معنوي في تركيز الزنك في أمصال دم المجموعة الفعالة من المرض عند مقارنتها مع المجموعة غير الفعالة . كذلك بينت هذه الدراسة إن هناك ارتباط ايجابي بين فترة المرض وتركيز عنصر النحاس بينما كان هناك ارتباط سلبي بين تركيز عنصر الزنك وفترة المرض.

بينت نتائج هذه الدراسة إن هناك أدلة تشير إلى دور كل من النحاس والزنك في التغيرات الباثولوجية التي تحدث عند المرضى المصابين بالتهاب المفاصل الرثوي وكذلك تؤثر هذه العناصر في درجة تطور المرض.

Introduction:

Rheumatoid arthritis is a progressive autoimmune disease of unknown etiology occurring in approximately 3% of the population. It is characterized by inflammation of the membranes lining the joint, which in turn causes pain, stiffness, redness and swelling. Bone and cartilage become damaged and the joint eventually loses its shape which contributes to pain and stiffness. The synovium becomes inflamed and painful and usually affects hands, knees, ankles and feet (1)

The causes of rheumatoid arthritis are not known. It is theorized that endocrine infectious, or genetic factors may play a role (2). This disease progresses from inflammation of the synovial

membrane to destruction of the joint capsule and bone, and finally to bony ankylosis and immobility. Additionally, This disease most affects women 2-3 times more often than men. Average age at onset is 35 years but the disease can also occur in children (3).

Copper is one of a relatively small group of metallic elements that are essential to human health. These elements, along with amino and fatty acids as well as vitamins, are required for normal metabolic processes. However, as the body cannot synthesize copper, the human diet must supply regular amounts for absorption (4, 5); Copper combines with certain proteins to produce enzymes that act as catalysts to help a number of body functions. Some help provide energy required by biochemical reactions. Others are involved in the transformation of melanin for pigmentation of the skin and still others help to form cross-links in collagen and elastin and thereby maintain and repair connective tissues. This is especially important for the heart and arteries. Research suggests that copper deficiency is one factor leading to an increased risk of developing coronary heart disease(6). Zinc is very important ,as it plays a crucial role with hundreds of process in the human body .The most prominent functions include support of the immune system ,joints ,tissue , along with aiding in cell growth .Additionally this metal plays important role in the function of most our senses ,which includes the ability to taste ,smell ,and also sight (7,8).

The mechanism(s) by which cells play role in rheumatoid 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), tumournecrosis factor-a (TNF-a) and interleukin-6 (IL-6)(9,10). These substances are immunocytokines liberated dose-dependent mode, mostly by activated macrophages, in response to several stimuli, including exercise, trauma, stress, or infection (11).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 (12). The purpose of the present study was to investigate the status of essential trace elements Zn^{++} and Cu^{++} concentrations in patients with RA.

Materials and methods:

Totally 34 subjects were enrolled in the study, 22 patients (16 F, 6 M; mean of ages= 45.67 ± 12.20), and 12 healthy individuals (mean of ages = 35.85 ± 9.65). Distribution of cases in regard with the activity and duration of the disease were shown in the table (1), (2) respectively. 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 (13). On the other hand the term "disease in remission" required that the patient had no reticular involvement and symptoms, and had a morning stiffness below 15 minutes and ESR less than 20 mm/hour. According to the foregoing definitions, 14 patients had active disease and 8 were under remission. After overnight fasting, total 10 ml venous blood was with drawn. 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 Cu and Zn levels by Perkin-Elmer atomic absorption spectrophotometry. ESR estimation was performed by Westergren technique in each patient. Results were documented in terms of $\mu\text{mol/L}$. The results of the analysis were confirmed by student's t- test and linear regression analysis was used to evaluate the correlation among parameters. All results were expressed as mean values \pm SD; statistical significance was defined as $p < 0.05$.

Table (1): Distribution of cases in regard with sexes and the activity of disease.

| Subjects | Female (n /age means) | Male (n /age means) |
|---------------|--------------------------|------------------------|
| Active RA | 10 (43.67±9.02) | 4 (47.20±14.20) |
| Remitional RA | 6 (43±11.04) | 2 (53.00±8.16) |
| Total | 16 (45.22±13.90) | 6 (49.33±9.98) |

Table (2): Distribution of cases in regard with duration of RA

| Duration (months) | n |
|-------------------|---|
| 6 | 5 |
| 12 | 7 |
| 24 | 4 |
| 36 | 2 |
| 48 | 2 |

Results and discussion:

Serum levels of copper and zinc were measured in 22 patients with rheumatoid arthritis (RA) and 12 healthy subjects. Patients with RA divided in two groups ;active RA 14 patients ,inactive RA were 8 patients .The results were analyzed using students t-test ,at evaluated serum copper found there were significant (P<0.01) when compared to control and patients with RA .However ,serum Zn concentration were lower in RA patients according to the control subjects(P<0.05).As shown in the table (3).

Table (3): Serum Cu⁺⁺ and Zn⁺⁺ levels in patients with RA and healthy subjects .

| Trace elements | Patients N=22 | Controls N=12 | P value |
|---------------------------|------------------|------------------|---------|
| | Mean ±SD | Mean ±SD | |
| Cu ⁺⁺ (µmol/L) | 29.06±3.78 | 20.74±4.30 | <0.01 |
| Zn ⁺⁺ (µmol/L) | 7.78±4.41 | 13.55±6.00 | <0.05 |

Additionally, serum cu⁺⁺ levels were (32.41 ±4.98 µmol/L) in patients with active RA ,and (28.52±3.92 µmol/L) in patients with inactive RA. But there was no significant difference between two groups (P>0.05).Serum Zn⁺⁺ levels were (10.21±6.01µmol/L) in patients with inactive RA, and (4.98±1.05µmol/L) in patients with active RA, there was significant difference between two groups (P<0.05), as shown in the table (4).

Table (4): Serum Cu⁺⁺ and Zn⁺⁺ levels in patients with active RA and inactive RA patients.

| Trace elements | active RA N=14 | inactive RA N=8 | P value |
|---------------------------|-------------------|--------------------|-----------|
| | Mean ±SD | Mean ±SD | |
| Cu ⁺⁺ (µmol/L) | 32.41±4.98 | 28.52±3.92 | N.S >0.05 |
| Zn ⁺⁺ (µmol/L) | 4.98±1.05 | 10.21±6.01 | <0.05 |

In this study we found a spectacular relationship between factors determining the activity of the disease such as ESR , the range was (28 -130 mm/hour) and the mean (63.4±28.6 mm/h) and alkaline phosphatase activity range was (160-497 IU/L) and the mean (243± 54.73 IU/L). Previous studies maintained a heightened alkaline Phosphates level in patients with active RA due to increased bone metabolism in these patients (14-17). In this study such correlation is an evidence that bone destruction is a continuous process in active disease processes.

In our study , the correlation between serum copper and zinc levels with duration of disease were examined statistically using the linear regression analysis .Positive correlation between serum Cu⁺⁺ levels and disease duration was observed (r : 0.489 , P<0.001).Correlation between serum cu⁺⁺ levels and disease duration was negative (r :0.248 P<0.05) table (5).

Table (5): Results of unvaried analysis of copper and zinc serum levels and duration of rheumatoid arthritis disease.

| Subjects | r | P value |
|----------|-------|---------|
| Copper | 0.489 | <0.001 |
| zinc | 0.248 | <0.05 |

Our study showing that patient with RA have markedly elevated serum Cu⁺⁺ levels compared with normal subjects . 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 (18,19). 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 (20, 21,22).

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. As we noted in our study no correlation exists between active and inactive patients in terms of serum Cu⁺⁺ levels. We found meaningful relationship between serum Cu⁺⁺ levels and duration of disease. These data agreement with other recent reports (23, 24).

Additionally 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 (25). Continuous hepatic synthesis of acute phase reactants during an inflammation is held responsible for this pathologic distribution (26). Factors mediating the synthesis of acute phase reactants are Cortizol and Cytokins (IL-1, IL-6, TNF). These mediators induce the synthesis of metallothioenins and overburdening of Zn⁺⁺ in the liver (26, 27, 28). This, in turn, 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 (20).This finding shows that serum Zn⁺⁺ decreases result from such several factors as trauma, infections, and medical treatment of the disease (29).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 (30).

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

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