Serum Copper as a Marker of Disease Activity in Rheumatoid Arthritis

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ABSTRACT

Introduction: Copper is an important trace element for normal growth and development of the body. It is also essential for maturation of collagen tissues. The purpose of the study was to estimate the serum copper levels in rheumatoid arthritis patients and to see its association with the various parameters of disease activity.

Materials and Methods: The study was carried out among 50 diagnosed rheumatoid arthritis patients (25 each of active disease & remission patients) and 50 age and sex matched controls. Fasting blood sample was collected for estimation of serum copper, haemoglobin level and ESR in the subjects.

Results: Mean serum copper level in the case group was found to be significantly higher than that of the control group (p-value<0.001). This increase of copper level was more in active disease than those with remission (p-value < 0.0001). A significant positive correlation was found between serum copper level and ESR, serum copper level and morning stiffness and a negative correlation was found between serum copper level and haemoglobin level in rheumatoid arthritis patients.

Conclusion: In rheumatoid arthritis patients, serum copper level may be used as an additional biochemical marker for estimation of disease activity.

Keywords: Arthritis, ESR, Haemoglobin, Morning stiffness

INTRODUCTION

Rheumatoid arthritis is a chronic disease with a wide variety of systemic manifestations including persistent inflammatory synovitis, usually involving peripheral joints in a symmetric manner. Synovitis leads to cartilage damage and bone erosions and subsequently leads to changes in joint integrity. Clinical presentation varies from mild oligoarticular illness to relentless progressive polyarthritis with significant functional impairment [1].

According to the modern concept, the pathogenesis of RA is a complex process involving synovial cell proliferation and fibrosis, pannus formation and cartilage and bone erosion [2-4]. In India, the prevalence of rheumatoid arthritis in rural population is 0.7% according to Malviya et al., [5].

American College of Rheumatology developed revised criteria in 1987, for classification of RA, which demonstrate a sensitivity of 91-94% and a specificity of 89%. These criteria can be used as guidelines for establishing diagnosis. According to this revised criteria, four of seven criteria are required to classify a patient as having rheumatoid arthritis (RA). The seven criteria includes: morning stiffness, arthritis of three or more joint areas, arthritis of hand joints, symmetric arthritis, rheumatoid nodules, serum rheumatoid factor and radiographic changes [1,6].

Foods high in copper and copper bracelets were thought to be beneficial in treating arthritic conditions. In 1945, patients with rheumatoid arthritis were shown to exhibit higher than normal serum copper levels [7]. Copper complexes were used in the treatment of RA in the 50's and 60's [8]. Even the time-tested copper bracelet was eventually shown to be an effective anti-inflammatory, due to the absorption of copper through the skin. In fact, the main copper containing enzyme, ceruloplasmin, is significantly elevated in inflammatory conditions and has anti -inflammatory activity [9,10]. Most of the copper found in human body is in protein bound form like ceruloplasmin, copper-albumin, metallothionines, copper containing enzymes like cytochrome c oxidase, superoxide dismuatse, lysyl oxidase etc. It plays a role in the production of haemoglobin, (the main component of RBCs), myelin (the substance that surrounds nerve fibres) and melanin (a dark pigment hair and skin). Copper deficiency significantly lower the activity of the copper dependent enzymes due to which determination of copper required by the body is important [11].

Many studies are available which found variable results regarding serum copper levels in RA patients [12-15].

Keeping the above points in mind, we made an attempt to do a study on serum level of copper in RA patients and to see the association between the markers of disease activity and the level of serum copper in RA, if any.

MATERIALS AND METHODS

This hospital based cross-sectional study was carried out among 50 cases of rheumatoid arthritis that were diagnosed according to the revised criteria outlined by American College of rheumatology, 1987. Fifty age and sex matched subjects were taken as control.

Diagnosis of RA was made as per ACR (American college of Rheumatology) criteria for clinical remission of rheumatoid arthritis [6]. RA patients not in clinical remission were taken as having active disease. Conditions found to increase copper levels-any other acute inflammatory conditions, renal, hepatic diseases, urinary tract infections, leprosy, malignancies, pregnant women, patients on oral contraceptive pills, patients wearing copper bracelets, ornaments or patients using copper utensils for cooking and feeding were excluded from the study. Also care was taken to exclude patients on disease modifying anti rheumatic drugs (DMARDS) or any other antibiotic.

Fasting blood samples were collected for estimation of serum copper, haemoglobin level and ESR. Serum copper level was estimated within 2 to 3 hours of blood-collection, in the department of biochemistry, by simple and sensitive colorimetric method.

Copper, released from ceruloplasmin in an acidic medium, reacts with Di-Br-PAESA to form a orange-red coloured complex. Intensity of the complex formed is directly proportional to the amount of copper present in the sample [16].

Erythrocyte Sedimentation rate (ESR) and haemoglobin levels were estimated by Westegrens method and acid haematin method respectively.

STATISTICAL ANALYSIS

A database was constructed on Microsoft Excel 2007, and statistical analysis was done. Students t-test and Pearson's correlation tests were done.

RESULTS

Morning stiffness, haemoglobin, ESR and serum copper levels were measured in control and case groups and means and standard deviations were calculated [Table/Fig-1].

In the study, we found higher incidence of RA in females (76%) than in males (24%). The ratio was found to be approximately 1:3.

Mean serum copper level amongst male and female cases was 172.91µg/dl and 176.71 µg/dl respectively. There was no significant difference (p-value >0.05) in serum copper level between the two groups, as evaluated by applying students t-test.

The mean serum copper level in the case group was significantly higher than in control group (p-value<0.001). On comparing 25 cases of active disease with 25 cases of disease in remission, the mean serum copper was found to be 200 ± 23.18 µg/dl and 151.6 ± 21.15 µg/dl respectively, thus showing a highly significant difference in the two groups (p-value < 0.0001) [Table/Fig-2].

ESR was found to be elevated in cases with a mean level of 47.72 ± 36.80 mm at the end of 1st hour, which is very highly

Parameters	Controls Mean ± SD	Active disease (Mean ± SD)	Disease in remission (Mean ± SD)
Serum Cu (µg/dl)	111.16 ± 24.95	200 ± 23.18	151.6 ± 21.15
ESR (mm)	21.26 ± 15.77	80 ± 25	17.36 ± 6.38
Haemoglobin(g/dl)	10.86 ± 2.17	8.41 ± 1.41	9.42 ± 1.45
Morning stiffness	…….	40min aprox.	<12min

[Table/Fig-1]: Parameters measured in the study group
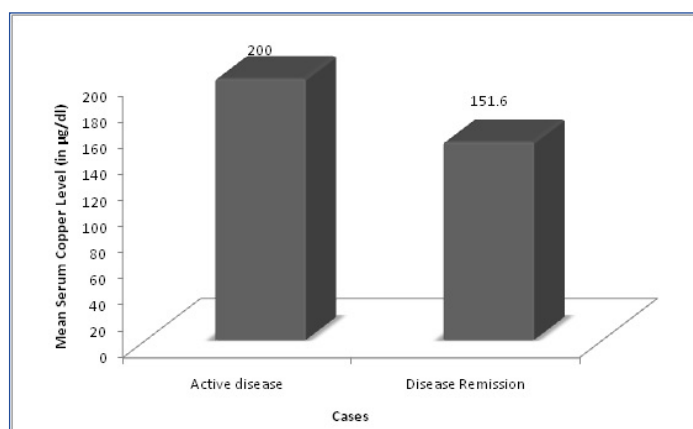
[Table/Fig-2]: Bar diagram showing mean serum copper levels in groups with active disease and disease in remission

Cases	No of patients	Correlation Coefficient	p-value
ESR	50	0.73	<0.001
Haemoglobin	50	-0.45	<0.01
Morning Stiffness	50	0.46	<0.01

[Table/Fig-3]: Relation between Serum Copper Concentration and the following markers of disease activity

significant (p-value<0.001), whereas haemoglobin was found to be significantly lower in cases as compared to controls (p-value<0.01). An extremely significant difference in ESR at the end of the 1st hour (p-value<0.001) and, haemoglobin levels (p-value<0.0165) were found between the two groups i.e. cases of active disease and cases of disease in remission.

A very highly significant positive correlation (r=0.73, p<0.001) has been observed between Serum copper level and ESR and significant positive correlation (r=0.46, p<0.01) also has been noticed between serum copper level and morning stiffness. On the other hand, a significant negative correlation (r= -0.45, p<0.001) has been observed between serum copper level and haemoglobin.

DISCUSSION

The distribution of cases were mostly clustered in the age group of 27-31 years (16%) followed by the age group of 37-41 years (14%) and 52-56 years (14%). 60% of the cases were between 25-50 years of age. Only 2 cases (4%) were seen in the age group beyond 61 years. The finding conforms to the observation made by ARA (1987) who reported the maximum age incidence between 25-30 years [1]. Similar age incidence was observed by Malaviya et al., in a study conducted by a house-house survey of a population near Delhi in 1993 [5].

In the present study there were 12 (24%) male cases and 38 (76%) female cases and the male: female ratio was 1:3.01. The findings coincide with those of ARA which states the ratio of male and female cases as 1:3. Kiss, lovei, et al., found similar results in a survey on rheumatoid arthritis patients in Hungary [17].

Serum copper level in this study was found to be significantly elevated in the cases as compared to control group. There was also highly significant difference in the serum copper level between active disease group and group in remission (p-value < 0.0001). These findings are similar to that of studies done by Xin L et al., Strecker D et al., and Shatha Rouf Moustafa et al., [12-14]. It might be attributed to the release of copper from its stores predominantly liver to maintain its increased requirement by lysyl oxidase, superoxide dismutase, glycylhistidine complex etc. [11]. Also, ceruloplasmin itself is said to have anti-inflammatory role. So an additional role is perhaps played by ceruloplasmin which is increased in rheumatoid arthritis as an acute phase protein.

The ESR was seen to be significantly elevated in cases as compared to control group. Among the case group again, patients in active disease, had a much higher level of ESR at the end of 1st hour as opposed to patients with disease in remission. The difference in the mean was extremely significant. Similar results were found by O. Cagayan et al., and Grennan D et al., [18,19]. In RA, fibrinogen, alpha 2 macroglobulin and immunoglobulins are increased which in turn helps in aggregation of RBCs and hence increased in ESR level [20].

Mean haemoglobin level in 50 patients of rheumatoid arthritis was significantly lower as opposed to that of control groups. Again those with active disease had a significantly lower haemoglobin level than in those with disease in remission (p<0.05), which implies that severity of Anaemia correlates with severity of the disease. Anaemia in rheumatoid arthritis may be due to decreased utilization of iron, reduced erythropoietin levels, premature destruction of red blood cells, NSAID associated Anaemia [21].

Very highly significant positive correlation (r=0.73, p<0.001) was observed between serum copper level and ESR and significant positive correlation (r=0.46, p<0.01) was also found between serum copper level and morning stiffness. On the other hand, a significant negative correlation (r= -0.45, p<0.001) has been observed between serum copper level and haemoglobin [Table/Fig-3].

AG White et al., reported that serum copper level can be used as an index of erosive activity in rheumatoid arthritis [22]. Highly significant

correlation was found between serum copper level and composite activity index criteria which among others includes haemoglobin, ESR, morning stiffness. (r=0.73, p<0.001).

LIMITATION

The limitation of our study includes less number of subjects involved in the study. Also, since liver is the store house of copper, estimation of hepatic tissue content of copper would have been a better indicator of actual amount of total body copper which could have helped in giving a direction towards treatment of rheumatoid arthritis, by supplementation of copper. Follow up study of disease activity with supplementation of copper could have revealed the role of copper more elaborately.

CONCLUSION

In the study we found that, serum copper level raises with the disease activity in RA; hence can be used as an alternative indirect marker to assess disease activity as well as severity in addition to the existing parameters.

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