Assessment of mineral status (Zn, Cu, Mg and Mn) in rheumatoid arthritis patients in Chandigarh, India

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Abstract

Past studies suggest that the deficiencies of Zn and Cu in patients of rheumatoid arthritis (RA) occur in response to chronic inflammation. However, therapeutic supplementation of Zn or Cu in RA yielded contradictory results. To find out the role of dietary factors, the present epidemiological study was carried out on the mineral status (Zn, Cu, Mg, Mn) in RA patients versus healthy subjects of the Chandigarh, India population where the consumption of Zn from dietary sources is more than its recommended dietary allowance (15-20 mg/day). The results of this study show reduced serum Zn, elevated serum Cu, Mg and Mn levels along with their increased urinary excretions and decreased hair concentrations in RA patients than healthy subjects. The significantly positive correlation (r) between inflammatory markers such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and negative between serum CRP, ESR vs. hair minerals {r = -0.897**, -0.944** (Cu), -0.984**, -0.978** (Mg), 0.777**, 0.716** (Mn) and -0.845**, -0.846** (Zn)}, and positive between serum CRP, ESR vs. urine minerals {r = 0.833**, 0.870** (Cu), 0.790**, 0.700** (Zn), 0.874**, 0.830** (Mg), 0.462**, 0.483** (Mn)} and negative between serum CRP, ESR vs. hair minerals {r = -0.961**, -0.970** (Cu), -0.897**, -0.913** (Zn), -0.959**, -0.958** (Mg), -0.535**, -0.484** (Mn), **p<0.0001} were reported. The data suggest that RA patients were unable to retain Zn in their tissues (serum and hair) and experienced excessive loss along with other metals through urinary excretions in spite of higher Zn consumption through the food chain.

Materials and Methods

Subjects

The present study was conducted on RA patients of the Chandigarh, India population. Two hundred (200) female subjects were chosen in the present study, and they were grouped as normal healthy subjects in Group-A (control) and RA patients in Group-RA, matched with number, age and gender (Table 1). Mean age and body weights were recorded and measured in each group including the duration of the disease activity in Group-RA (Table 1).

Collection of patient data

The personal and medical history along with relevant details of RA patients was recorded in the present study after formal consent was obtained through a questionnaire as per guide lines of the Ethics Committee. The information requested included the patient’s sex, age, samples taken (hair, blood serum and urine), place of residence, occupation and type of eating habits. Samples were collected from the Orthopediatric Clinic, Sector-16D, Chandigarh (under the guidance of Dr. H. C. Gupta, Orthopedics and General Surgeon, Former Head of Department of Surgery and Orthopedics, General Hospital, Sector-16D, Chandigarh) and Mr. K. L. Kapur (Tarun Clinic, Sector-46 C, Chandigarh) for providing the samples. Regarding the contribution of each author in the present study, the first author (corresponding author) has supervised the present research work and second author has pursued it (contributing author). The Authors report no conflicts of interest between the authors and funding agency.

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Introduction

The mortality rate in patients of rheumatoid arthritis (RA) is high owing to their exposure to greater risk for cardiovascular diseases, myocardial infarction (heart attack) and stroke. The role of Zn (Zinc) and Cu (Copper) in RA, a chronic inflammatory disease is of great interest as they are the co-factor of important enzymes involved in collagen and bone metabolism, the antioxidant defense system and the immune system. In the previous studies, the development and progression of RA was suggested due to marginal deficiencies of Zn and Cu based on their serum levels. The alteration of these two trace elements has been linked to inflammatory response and has not been suggested to depend upon dietary factors which are poorly understood in RA. The previous studies have reported low dietary intakes of Zn, Cu and Mg in RA patients resulting in decreased activities of antioxidant enzymes including Cu-Zn superoxide dismutase, glutathione peroxidase and catalase. Therefore, in many clinical trials, therapeutic supplementation of Zn or Cu in RA patients has been investigated which yielded contradictory results.

During the past two decades, there has been a rise in the consumption of higher Zn either from Zn fortified foods as in the USA or from vegetables and meat foods stuffs (40 mg/kg or more in above ground vegetables and 120 mg/kg or above in underground vegetables) as in Chandigarh, India. Excessive intake of Zn has been reported to induce Cu, Mg, Mn, Fe and Se deficiencies due to their antagonistic effects and also it induces inflammatory response through cytokine production by morpho-nuclear cells which is a consistent feature in RA. Therefore, the present epidemiological study was carried out to estimate minerals in RA patients of this region of higher Zn consumption from their diets for a better understanding of mechanisms involved in this disease.

Materials and Methods

Subjects

The present study was conducted on RA patients of the Chandigarh, India population. Two hundred (200) female subjects were chosen in the present study, and they were grouped as normal healthy subjects in Group-A (control) and RA patients in Group-RA, matched with number, age and gender (Table 1). Mean age and body weights were recorded and measured in each group including the duration of the disease activity in Group-RA (Table 1).
Sampling of urine for mineral analysis

The morning urine samples (spot urine) were collected in sterilized metal free vials. The samples were collected in collection vials from each healthy subject as well as from RA patients after every three days for 3 times and stored in the metal free vials (using conc. HNO3). The timing for the spot urine sample was standardized. The urine samples in plastic vials were stored at 2-4 °C for the analysis of minerals. Long storage of both serum and urine samples was avoided in the present investigations.

Collection of hair samples

For the collection of hair samples, the healthy subjects and RA patients were asked to wash their hair thoroughly with distilled water and medicated soap (dettole) devoid of any metal contamination, followed by drying with a clean towel to remove any external contamination. Then, with the help of clean stainless steel scissors, hairs from the neck region were collected in the plastic polythene bags.

Washings

The hair samples were scraped and cut with stainless steel scissors and were cleaned of dust particles with non-ionic detergent Triton X-100 following the standardized washing procedures. Subsequently, the hair samples were soaked in acetone to remove external contamination, then in alcohol, rinsed five times with distilled deionized water (TDW). The ash so formed was dissolved in 6 mL of 10 mM nitric acid and filtered through ash-free filter paper before analysis. They were analyzed for Zn, Cu, Mg and Mn levels on the atomic absorption spectrophotometer (Electronic Corporation of India Limited, Hyderabad, AAS 4139) using hollow cathode lamps (213.9 nm, 324.8 nm, 285.2 and 279.5 nm for Zn, Cu, Mg and Mn, respectively) in triplicates with dilutions. Standards for Zn, Cu, Mg and Mn (Sigma) were prepared in triple distilled deionized water (TDW).

Statistical analysis

The data was subjected to statistical analysis applying Student’s t-test and analysis of variance (ANOVA). Results were expressed as mean ± standard error mean (SEM) at the significance levels p<0.001 (highly significant), 0.01 (significant) and 0.05 (almost significant) in the tabular form. Pearson’s correlation analysis was utilized to determine the relationship between inflammatory markers serum CRP, ESR with mineral levels (Cu, Zn, Mg and Mn) in the blood serum, urine and hair, respectively. Statistical significance level for Pearson’s correlation was set at p<0.01 (highly significant). SPSS for Windows (release 10.0, Panjab University, Chandigarh) was used for statistical analysis in the present study.

Results

Body weights

The mean body weights recorded were less in RA patients than in healthy subjects (Table 1).

Cu, Zn, Mg and Mn status in RA patients vs. healthy subjects

A highly significant increase in serum Cu, Zn, Mg and Mn levels along with (Table 2, p<0.001) significant reduction in serum Zn (Table 2, p<0.05) were evaluated in RA patients. Highly significant increase in urinary Cu, Mg and Mn concentrations (Table 2, p<0.01, 0.01) along with significant (almost) rise in urine Zn (Table 2, p<0.05) were recorded in group-RA. Hair mineral data showed highly significant reductions in Cu, Zn, Mg and Mn concentrations in RA patients compared with those of healthy subjects (Table 2, p<0.001).

Correlations (r)s between minerals (serum, urine and hair) vs. serum CRP and ESR: Significant (r)s were evaluated between serum minerals with serum CRP, ESR which was found to be positive for Cu, Mg and Mn but negative for serum Zn in RA patients vs. healthy subjects, respectively [Serum CRP vs. serum minerals: r = 0.973** (Cu), 0.978 ** (Mg), 0.777** (Zn) and -0.845** (Mn), **p<0.0001; ESR vs. serum minerals: r = 0.978** (Cu), 0.979** (Mg) and 0.716** (Mn) and -0.846** (Zn), **p<0.0001]. Similarly, significant positive (r)s were evaluated between urinary minerals (Cu, Zn, Mg and Mn) vs. serum CRP, ESR and significant negative (r)s between hair minerals vs. serum CRP and ESR in RA patients vs. healthy subjects [Serum CRP vs. urine minerals: r = 0.835** (Cu), 0.790** (Zn), 0.874 ** (Mg), 0.462** (Mn), **p<0.0001; ESR vs. urine minerals: r = 0.870** (Cu), 0.700** (Zn), 0.830** (Mg) and 0.483** (Mn), **p<0.0001; serum CRP vs. hair minerals: r = -0.961** (Cu), -0.897** (Zn), -0.959** (Mg), -0.535** (Mn), **p<0.0001; ESR vs. hair minerals: r = -0.970** (Cu), -0.913** (Zn), -0.958** (Mg), -0.484** (Mn), **p<0.0001, p<0.01 (level of significance)] respectively.

Discussion

The results of the present study showed a higher Cu, Mg and Mn, and lower Zn levels in the serum and significantly lower concentrations in the hair of RA patients than those of healthy subjects (Table 2). Such a change in mineral concentrations in the human tissues and fluids plays important roles in health and disease conditions as they are the important
components of the antioxidant enzyme defense system.\textsuperscript{10,12,23}

The hypozincemia and hypercupremia observed in RA patients were correlated negatively and positively with pro-inflammatory markers i.e. serum CRP and ESR, respectively (Table 2) and are consistent with previous reports.\textsuperscript{4,9,10} This alteration in Zn and Cu metabolism in blood serum has been suggested to be influenced by chronic inflammatory response causing their accumulation\textsuperscript{14} in many body compartments and in the inflamed areas as observed in laboratory animals, suggesting increased body requirement of Zn and Cu in RA patients.\textsuperscript{11} It is believed that the accumulation of Zn in Zn-containing protein metallothionein, a labile source of Zn for its distribution to inflamed joints\textsuperscript{22,23} and many other organs during inflammation,\textsuperscript{11} requires more Cu to activate yet unidentified metalloenzymes that are essential during the stress conditions leading to hypozincemia in RA.\textsuperscript{11,18,32-34} In spite of higher intake of Zn from the diet by RA patients in this region, the observed hypozincemia does not support the concept \textit{per se} but suggests its genetic predisposition and not the cause of the disease.

Because of the requirement of Zn and Cu in inflamed tissues, their bioavailability to the other tissue may decrease as observed in hair of RA patients compared with those of healthy subjects (Table 2) showing negative correlations between them.\textsuperscript{19,20} The present results, therefore, do not support Zn deficiency in its cause since the population of Chandigarh has been consuming a Zn-rich diet (40 mg/kg or more in above ground vegetables and 120 mg/kg or above in underground vegetables)\textsuperscript{23} for the last two decades and reaffirm the finding of Craing et al.\textsuperscript{24} who suggested that RA patients were not Zn deficient. Further, Zn supplementation trials in RA patients suggest that the contradictory results on Zn status.\textsuperscript{7,18,19}

It further finds support from the observations that hypozincemia in RA was not correlated with urinary Zn excretion (hyperzincuria) since there was little increase in urinary Zn excretion. Hypozincemia observed in RA patients may be attributed to cytokines such as interleukin-1 which rapidly synthesize CRP by hepatocytes into the circulation.\textsuperscript{36,37}

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group-A (Control)</th>
<th>Group-RA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Cu*</td>
<td>0.047±0.01</td>
<td>1.740±0.02c</td>
</tr>
<tr>
<td>Serum Zn*</td>
<td>0.338±0.01</td>
<td>0.296±0.01c</td>
</tr>
<tr>
<td>Serum Mg*</td>
<td>0.396±0.01</td>
<td>1.700±0.01c</td>
</tr>
<tr>
<td>Serum Mn*</td>
<td>2.230±0.07</td>
<td>2.880±0.02c</td>
</tr>
<tr>
<td>Urine Cu*</td>
<td>3.980±0.18</td>
<td>8.000±0.27c</td>
</tr>
<tr>
<td>Urine Zn*</td>
<td>0.241±0.01</td>
<td>0.329±0.06c</td>
</tr>
<tr>
<td>Urine Mg*</td>
<td>0.590±0.05</td>
<td>1.790±0.06c</td>
</tr>
<tr>
<td>Urine Mn*</td>
<td>1.140±0.20</td>
<td>1.450±0.07c</td>
</tr>
<tr>
<td>Hair Cu*</td>
<td>186.84±0.42</td>
<td>104.28±2.07c</td>
</tr>
<tr>
<td>Hair Zn*</td>
<td>47.12±0.36</td>
<td>33.12±0.83c</td>
</tr>
<tr>
<td>Hair Mg*</td>
<td>32.28±0.21</td>
<td>17.96±0.22c</td>
</tr>
<tr>
<td>Hair Mn*</td>
<td>97.12±5.65</td>
<td>73.88±0.94c</td>
</tr>
</tbody>
</table>

\*mg/dL; \textsuperscript{10}mg/g of tissues; \(p<0.001, \textsuperscript{11}<0.01; \textsuperscript{12}<0.05\) (values of group-RA were compared with control group-A).

Correlated positively with CRP and ESR in RA.\textsuperscript{41} A highly significant reduction in hair Cu compared with hair Zn in RA patients confirms the presence of Cu deficiency as an outcome of hypercupremia and hypercuperuria, correlated negatively with serum CRP and ESR (Table 2). The generation of Cu deficiency in RA was suggested to be due to inflammatory response that has been found to alter Cu metabolism leading to reduced Cu status in them.\textsuperscript{42} Cu-supplementation (2 mg/day, four weeks) trials in RA patients have reported improved erythrocyte Cu-Zn superoxide dismutase activity confirming the presence of Cu deficiency in RA.\textsuperscript{42} The hypercupremia further leads to a decrease in HDL-c in RA patients resulting from Cu deficiency.\textsuperscript{4,9,10} This Cu-deficiency has been reported to weaken immune system function resulting in increased infections causing increased ESR in RA patients.\textsuperscript{4} Low HDL-c in RA further leads to inactivation of the immune system and increases the risk for inflammation in RA patients.\textsuperscript{47} Regarding the prolonged use of excessive Zn from the vegetable food stuffs in RA patients in this region, this may cause secondary Cu and Fe deficiencies due to their antagonistic interactions at intestinal uptake.\textsuperscript{40,42} This factor may contribute to greater reduction of Cu and Fe in the tissues of RA patients leading to more damage to the antioxidant enzyme defense system, inactivation of the immune system and reduction in Hb leading to increased oxidative stress and inflammatory response in the present study.\textsuperscript{15,19,21}

Lastly, the decrease in hair Mg and Mn levels in RA patients also showed reduced Mg and Mn status as evident from their increased serum and urinary concentrations (Table 2). These reports are contradictory to a previous report in RA patients\textsuperscript{47} which may be due to both inflammatory response along with excessive intake of Zn in RA patients as positive correlations were observed between serum and urinary Mg and Mn levels with CRP and ESR where both are required for optimal activity of the immune system.\textsuperscript{52,54}

### Table 2. Concentrations of minerals including Cu, Zn, Mg and Mn in the blood serum, urine and hair of RA patients (group-RA) and healthy subjects (group-A) in the population of Chandigarh, India. (Values are mean ± S.E.M. (n = 100)).

### Conclusion

From the present study, it may be possible to conclude that the cause-effect relationship in both inflammatory response and dietary factors influence the mineral status in RA patients. Inflammatory response was found to affect their intracellular and blood levels whereas dietary factors affect their mutual transport resulting in their deficiency and excess in the tissues. In RA patients of this region (Chandigarh), Zn deficiency can not be suggested since the increase in urinary Zn was of small fraction as compared to other minerals.

### References


