Original Article

Serum Zinc and Copper in Active Rheumatoid Arthritis: Correlation with Interleukin 1β and Tumour Necrosis Factor α

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Abstract: Serum zinc and copper levels and serum interleukin 1β (IL1β) and tumour necrosis factor α (TNFα) levels were evaluated in 57 female patients with active rheumatoid arthritis (RA) to investigate a possible role of IL1β and TNFα on zinc and copper homeostasis in RA. Serum zinc levels were significantly lower and serum copper levels significantly higher in RA patients when compared with osteoarthritis or asymmetrical psoriatic oligoarthritis patients and with normal controls. No differences were observed in serum IgM rheumatoid factor positive and serum IgM rheumatoid factor negative patients as regards serum zinc and copper concentration. In RA patients the erythrocyte sedimentation rate and acute-phase proteins correlated negatively with serum zinc and positively with serum copper. IL1β and TNFα were found to correlate negatively with zinc and positively with copper in RA patients. Lower levels of zinc may be due to an accumulation of zinc-containing proteins in the liver and in the inflamed joints in RA. Elevated serum copper levels seem to be linked to the increased synthesis of ceruloplasmin by the liver.

Keywords: Copper; Interleukin 1; Rheumatoid arthritis; Tumour Necrosis Factor; Zinc

Introduction

The role of zinc and copper in chronic inflammatory diseases is of interest because they are co-factors of important enzymes involved in collagen and bone metabolism [1,2], immune system function [3,4] and antioxidant protection [5].

Serum zinc was found to be reduced and serum copper increased in adult and juvenile rheumatoid arthritis (RA) and these variations appeared to be associated with the immune inflammatory rheumatoid process [5,6]. Changes in serum zinc and copper levels observed in RA patients led some investigators to hypothesise that a marginal deficiency in zinc and copper might contribute to the development of RA and to the progression of the disease itself [7,8].

Interleukin 1β (IL1β) and tumour necrosis factor α (TNFα) are products of stimulated monocytes and macrophages which can induce collagenase and other neutral proteases in synovial fibroblasts and chondrocytes, stimulate prostaglandin E₂ synthesis and osteoblast/osteoclast activity with resultant bone resorption and promote T and B cell responses [9]. Serum IL1β and TNFα were found to be elevated in RA patients and to correlate with disease activity [10,11].

In this study we have analysed possible correlations between serum zinc and copper levels and serum IL1β and TNFα levels in patients with active RA to investigate a possible role of IL1β and TNFα on zinc and copper homeostasis in RA. Furthermore, we have correlated serum zinc and copper with various laboratory parameters and inflammation.

Materials and Methods

Fifty-seven female patients affected by RA according to the 1987 American Rheumatism Association criteria [12] were studied. The mean (±SD) age of the patients was...
52.2 ± 3.6 years and the mean disease duration was 3.4 ± 2.4 years. According to the functional ARA classification the patients performed their usual activities with moderate difficulty or were limited in their usual occupation (Steinbrocker functional class II or III). No patient was wholly incapacitated or unable to perform self-care.

All the patients had active disease defined by the following criteria: erythrocyte sedimentation rate (ESR) of at least 30 mm/h, six or more tender joints, three or more swollen joints and morning stiffness of at least 30 min duration. Thirty patients were IgM rheumatoid factor positive.

None of the patients had been treated with steroids, immunosuppressives or penicillamine in the 3 months before the study. They all were receiving non-steroidal anti-inflammatory drugs (NSAIDs) (diclofenac sodium, 100 mg/day).

Twenty female patients affected by psoriatic arthropathy, oligoarticular clinical pattern, with a mean age of 50 ± 4.5 years receiving NSAIDs, 20 female patients treated by NSAIDs for knee or hip osteoarthritis and 20 healthy female subjects with a mean age of 53 ± 3.1 years constituted the control groups.

Blood samples were obtained using stainless steel needles following an overnight fast. ESR, fibrinogen, α1 acid glycoprotein, haptoglobin, ferritin, ceruloplasmin, immunoglobulins, and C3 and C4 complement components were determined by standard laboratory methods. Serum was stored at -40°C until analysis.

Serum zinc and copper were determined by colorimetric assays. The zinc colorimetric assay uses 2-(5-bromo-2-pyridilazo)-5-(N-propyl-sulphopropylammino)-phenol (WAKO, Japan). The sensitivity of the zinc colorimetric assay was 35 μg/dl; the intra-assay reproducibility coefficient of variation (CV) was 3% and the interassay CV was 10.5%. The copper colorimetric assay was 20 μg/dl; the intra-assay reproducibility CV was 5% and the interassay reproducibility CV was 10.5%.

IL1β and TNFα were measured by radioimmunoassay (RIA) systems (Amersham, UK), which use high specific activity 125I-IL1β and 125I-TNFα human recombinant tracers, together with highly specific and sensitive antisera. The sensitivity of the IL1β RIA was 2.2 fmol/ml. The intra-assay reproducibility CV was 3.2% and the interassay reproducibility CV was 7.1%. The sensitivity of the TNFα RIA was 5 fmol/ml. The intra-assay reproducibility CV was 6.3% and the interassay reproducibility CV was 11%.

Results are expressed as mean ± SD. Student’s t-test and Pearson coefficient were used for statistical analysis.

Results

Serum zinc levels were significantly lower and serum copper levels significantly higher in RA patients when compared with osteoarthritis patients (85.6 ± 21.9 vs 100.44 ± 14.61 μg/ml, p < 0.01 and 151.8 ± 33.2 vs 105 ± 17.05 μg/ml, p < 0.001, with psoriatic oligoarthrits patients (85.6 ± 21.9 vs 100 ± 19.53 μg/ml, p < 0.05 and 151.8 ± 33.2 vs 92 ± 21.86 μg/ml, p < 0.005) and with normal controls (85.6 ± 21.9 vs 108.1 ± 19.2 μg/ml, p < 0.001 and 151.8 ± 33.2 vs 177.8 ± 22.5 μg/ml, p < 0.001). In the same RA patients, serum IL1β and serum TNFα levels were significantly higher compared with osteoarthritis patients (38.6 ± 16.4 vs 23.2 ± 6.1 fmol/l, p < 0.01 and 127.3 ± 28.9 vs 43.5 ± 12.3 fmol/l, p < 0.01), psoriatic arthritis patients (38.6 ± 16.4 vs 28.3 ± 5.1 fmol/l, p < 0.05 and 127.3 ± 28.9 vs 64.6 ± 10.5 fmol/l, p < 0.01) and normal controls (38.6 ± 16.4 vs 20.4 ± 4.1 fmol/l, p < 0.01 and 127.3 ± 28.9 vs 36.2 ± 16.4 fmol/l, p < 0.001).

No differences were observed in serum IgM rheumatoid factor positive and serum IgM rheumatoid factor negative patients as regards serum zinc and copper concentration (87.33 ± 19.57 vs 84.06 ± 24.04 μg/ml and 147.37 ± 37.06 vs 155.86 ± 29.34 μg/ml; p = NS).

In RA patients, the ESR and acute-phase proteins such as C-reactive protein, α1 acid glycoprotein and fibrinogen correlated negatively with serum zinc and positively with serum copper. Serum copper positively correlated with ceruloplasmin, IgG and C3 (Table 1).

IL1β and TNFα were found to correlate negatively with zinc and positively with copper in RA patients. A positive correlation was observed between IL1β, TNFα and ceruloplasmin (Fig. 1).

No difference was found in serum IL1β and TNFα concentrations between IgM rheumatoid factor positive and IgM rheumatoid factor negative patients (RF positive: 36.8 ± 12.4 vs 40.1 ± 14.3 fmol/l, p = NS; RF negative: 128.8 ± 25.2 vs 125.5 ± 29.2 fmol/l).

No correlation was noted between serum zinc and copper levels and disease duration in RA patients.

Discussion

Serum concentrations of zinc and copper have been extensively studied in RA and a decrease of zinc as well as C-reactive protein, α1 acid glycoprotein and fibrinogen correlated negatively with serum zinc and positively with serum copper. Serum copper positively correlated with ceruloplasmin, IgG and C3 (Table 1).

Table 1: Correlation between activity indexes of disease and zinc and copper levels in patients with active rheumatoid arthritis

<table>
<thead>
<tr>
<th>Activity indexes</th>
<th>Zinc</th>
<th>Copper</th>
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<tbody>
<tr>
<td>ESR (mm/h)</td>
<td>−0.43</td>
<td>0.009</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)</td>
<td>−0.40</td>
<td>0.002</td>
</tr>
<tr>
<td>α1 Acid glycoprotein (mg/dl)</td>
<td>−0.38</td>
<td>0.004</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>−0.33</td>
<td>0.004</td>
</tr>
<tr>
<td>Ferritin (μg/l)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Ceruloplasmin (mg/dl)</td>
<td>NS</td>
<td>0.33</td>
</tr>
<tr>
<td>IgG (mg/dl)</td>
<td>NS</td>
<td>0.39</td>
</tr>
<tr>
<td>IgA (mg/dl)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>IgM (mg/dl)</td>
<td>NS</td>
<td>0.26</td>
</tr>
<tr>
<td>C3 (mg/dl)</td>
<td>NS</td>
<td>0.04</td>
</tr>
<tr>
<td>C4 (mg/dl)</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

ER, erythrocyte sedimentation rate; NS, not significant.