Predictive clinical and laboratory parameters for serum zinc and copper in rheumatoid arthritis

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SUMMARY Zinc and copper have important effects on T cell mediated immunity and on neutrophil function, but it is not known how the causes or effects, or both, of low serum zinc and high serum copper relate to the clinical picture of rheumatoid arthritis (RA). In this study serum zinc and copper determined by flame atomic absorption spectrometry and 30 other clinical, immunological, and laboratory parameters in 60 patients with RA were analysed by stepwise multiple linear regression analysis. Joint score index, rheumatoid factor titre, seropositivity, haemoglobin, and C reactive protein (CRP) were among the nine independent variables which together predicted 73% of the serum zinc variation. This suggests that there is an association between the immune-inflammatory rheumatoid process and the serum zinc concentration. CRP alone had only a 3% independent predicting value for serum zinc, however. This suggests the metallothionein mediated sequestration in the liver, induced by interleukin 1, is not an important explanatory factor in a cross sectional study of chronic inflammation. Furthermore, serum zinc did not have any predictive value at all for serum copper concentration. This does not support the hypothesis suggesting that serum zinc deficiency leads to high serum copper by inducing gastrointestinal metallothionein and high caeruloplasmin.

After iron, zinc and copper are the second and third most abundant trace elements in the human body. Zinc deficiency seems to enhance absorption of copper from food and to increase serum copper by inducing intestinal metallothionein and liver caeruloplasmin.¹ The simultaneous use of serum copper and zinc measurements has been advocated for the clinical assessment of certain diseases.² Serum zinc and copper may reflect the dietary content and availability of these elements, their absorption from the gastrointestinal tract, or excretion via urine and sweat.³ Furthermore, metallothionein dependent uptake and storage may also change serum zinc concentrations.⁴ An important regulatory factor may be activation of the phagocytic leucocytes, inducing them to secrete hormone-like substances and leading to an abrupt hepatic sequestration of zinc.⁴ More recently, interleukin 1 has been recognised as the primary mediator of the

acute phase response and has been suggested as a possible cause of the changes of the serum trace elements associated with the acute phase response. In contrast with iron, serum zinc and copper have aroused relatively little interest.⁶ In this study we analysed possible associations between serum zinc or copper concentrations and various clinical variables in a group of patients with well characterised rheumatoid arthritis using stepwise multiple linear regression analysis.⁷

Patients and methods

PATIENTS

In 1985-6, at the Division of Rheumatic Diseases, Fourth Department of Medicine, Helsinki University, Finland, we studied 60 patients with classical or definite rheumatoid arthritis (RA) according to American Rheumatism Association (ARA) criteria.⁸ Patients receiving zinc or copper supplementation were excluded. The mean number of ARA criteria was 6.5 (range 5-8). Most patients had seropositive erosive RA, with erosions observed in

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55 patients, and a positive rheumatoid factor titre had been recorded in a Waaler-Rose or latex agglutination test at least twice during the course of the disease in 38 of the 60 patients. At the time of study, however, low or negative, slightly raised, and strong positive Waaler-Rose titres were recorded in 51, six, and three patients respectively, the corresponding figures for latex agglutination test being 51, eight, and one.

According to the functional classification of the ARA 45 of our patients had function adequate for normal activities despite the handicap of limited movement at one or more joints.⁹ Eight patients had a normal functional status, i.e., ARA class I, whereas seven were severely limited in their ability to perform normal occupational duties or usual self care. Incapacitated ARA class IV patients were excluded from this series.

The mean age of the patients was 53 (range 28–83) years and the mean duration of disease at the time of study 11 (range 1–52) years. Fifty six patients were women and four were men. The mean (SD) height and weight were 164 (6) cm and 63 (10) kg respectively. All patients were from the Greater Helsinki area.

Patients were asked about their dietary habits, including the consumption of fish, supplementation with selenium, zinc, copper, iron, and vitamins A, E, or C. Cigarette smoking was also recorded. Patients and physicians alike were also asked to make a comprehensive, subjective assessment of the condition of the patient on a five stage scale.

One of our patients was not receiving any drugs at the time of the study, and 26 patients were treated only with non-steroidal anti-inflammatory drugs (NSAIDs) or low dose prednisone (<6 mg/day), or both. Thirty two of the patients received a slow acting disease modifying drug: eight were receiving sulphasalazine, 15 aurothiomalate injections, six oral auranofin, and three hydroxychloroquine. In addition, three of the patients treated with gold also received hydroxychloroquine. Patients who had been receiving slow acting disease modifying drugs during the previous three months were recorded as being treated. One of our patients received 15 mg prednisone a day for her RA and asthma, but otherwise none of our patients was taking immunosuppressive or cytostatic drugs.

Fifty five patients had morning stiffness lasting for at least one hour. Inflammation was also assessed using standard erythrocyte sedimentation rate (36 (SD 23) mm/h), CRP (21 (22) mg/l), and haemoglobin (123 (17) g/l). To characterise the extent of the rheumatoid involvement a joint score index was recorded according to Kaarela's method,¹⁰ for which purpose involvement was measured by the extent of

swelling (except hips and cervical spine, where limitation of movement was recorded). Our patients had a mean (SD) score of 10 (5), the maximum Kaarela score being 23. Involvement of the 'small' peripheral joints (from the wrists to periphery or from the talocrural joint to the periphery, or both,) and of the 'large' joints (namely, elbows, glenohumeral joints, knee joints, hip joints, and cervical spine) was also recorded separately for statistical analysis. According to the occupation reported, 3.6% of patients were upper white collar personnel, 47.2% lower white collar personnel, 29.1% workers and 20.1% housewives, students, or pensioners whose earlier occupations were not known.¹¹ Control persons younger than 65 years of age were workers at research institutes in the Helsinki area. The over 65 year old controls lived in homes for the elderly.

ELEMENT ANALYSIS

Venous blood was obtained with stainless steel needles (Neolus, Terumo, Belgium) following an overnight fast. The serum was separated by centrifuging and stored at –20°C until analysis.

Zinc and copper concentrations were determined by flame atomic absorption spectrometry. A single dilution technique was used.¹² A Perkin-Elmer 5000 spectrometer was used for the atomic absorption determinations. To control analytical quality Sero-norm (Nyegaard & Co, Oslo, Norway) standard reference serum was analysed during every run.

STATISTICAL ANALYSIS

A BMDP-UCLA 1985 computer program was used for the statistical procedures. The relation between serum zinc and copper concentrations and the different clinical, laboratory, and other parameters was examined by stepwise multiple linear regression analysis in a forward manner. Table 1 shows the

Table 1 Variables used in stepwise regression

Serum zinc	ARA criteria
Serum copper	ARA functional classification
Age	ESR
Sex	Seropositivity
Weight	Erosions
Height	CRP
Occupation	Haemoglobin
Residence	Morning stiffness
Fish consumption	Waalser-Rose titre
Serum selenium	Latex titre
Smoking	Joint score index
Supplementation of	Type of joints affected (large, small)
iron	Disease duration
selenium	Assessment of the condition
vitamin A	by the patient
vitamin E	by the physicians
vitamin C	Drug treatment

variables considered. All the data in the text are presented as arithmetic means (SD). In addition, a *t* test (two sided; separate variance estimate) was used.

Results

SERUM ZINC AND COPPER CONCENTRATIONS
Serum zinc concentrations were lower in patients with RA (mean (SD) 10.2 (2.0) $\mu\text{mol/l}$) than in normal controls (11.9 (1.8) $\mu\text{mol/l}$; $p<0.001$; Fig 1). No age dependence was found in zinc values. The copper concentrations decreased in patients with RA with increasing age, while in the controls the opposite effect was found. In the group of patients less than 65 years old the mean copper concentration was higher (23.7 (5.3) $\mu\text{mol/l}$) in patients with RA

Fig. 1 Zinc and copper concentrations ($\mu\text{mol/l}$) in the serum of patients with rheumatoid arthritis and in healthy controls. Mean (SD).

than in controls (17.6 (4.9) $\mu\text{mol/l}$; $p<0.0001$). In old (>65 years) patients with RA the mean copper concentration was not significantly higher than that in controls (21.5 (3.3) $\mu\text{mol/l}$ v 21.0 (3.9) $\mu\text{mol/l}$). In a more detailed analysis we found that the illness duration was not dependent on age. Likewise, the joint score index was no higher among elderly patients. High copper concentrations were significantly ($p<0.01$) associated with treatment with slow acting disease modifying drugs, however. Treatment with NSAIDs or low dose prednisone, or both, was most often used among patients over 65 years of age ($p<0.01$).

PREDICTION OF VARIATION IN SERUM ZINC AND COPPER CONCENTRATIONS
Table 2 shows the independent variables with a predictive value for serum zinc concentration; nine variables together predicted 73% of the variation in serum zinc. The most prominent predicting variables were the joint score index, Waaler-Rose titre and haemoglobin. Table 2 shows six variables which predict the variation in serum copper. The best predictive independent variables for copper were age and erythrocyte sedimentation rate (ESR).

Discussion

Different variables reflecting the extent and severity of RA were good predictors for serum zinc concentration. The joint score index was the most significant independent variable, predicting 39% of variation in serum zinc, and, conversely, the variation in serum zinc predicted 36% of the joint score index. Rheumatoid factor titre, seropositivity, haemoglobin, and CRP were also among the nine variables which together predicted 73% of the serum zinc variation. Our results thus indicate an association between the extent and severity of the immune

Table 2 Independent variables predicting zinc and serum copper values. Analysis using BMDP-UCLA 1985 program in forward stepwise multiple linear regression analysis

Zinc			Copper		
Independent variable	Cumulative predictive value (%)	p Value	Independent variable	Cumulative predictive value (%)	p Value
Joint score index	39	<0.001	Age	20	<0.002
Waaler-Rose titre	44	<0.01	ESR	30	NS
Haemoglobin	49	<0.01	Selenium	36	NS
Age	55	NS	Smoking	42	NS
Living area	60	<0.01	Hydroxychloroquine treatment	46	NS
Seropositivity	64	<0.05	ARA criteria	52	NS
CRP	67	<0.01			
Vitamin E supplementation	70	<0.01			
Hydroxychloroquine treatment	73	NS			

inflammatory rheumatoid process and serum zinc concentration.

It was noteworthy that CRP, though a good measure of the acute phase response, had an independent predictive value of only 3%. Therefore, metallothionein mediated sequestration in the liver, induced by interleukin 1, is not an important explanatory factor in the cross sectional study of chronic inflammation,^{5 6} which is in contrast with the short term, acute phase response caused, for instance, by microbial invasion.

Our results confirm those of earlier work, which demonstrated low serum zinc and increased serum copper in rheumatoid arthritis.¹³⁻¹⁶ Serum copper had no independent predictive value for serum zinc, however, and serum zinc had none for serum copper. Therefore our work suggests that mechanisms other than gastrointestinal metallothionein and serum caeruloplasmin, both induced by low serum zinc, are responsible for these opposite changes in zinc and copper concentrations. Our results suggest rather that low serum zinc and high serum copper in RA reflect a myriad of changes in various mediators and body constituents (see also Table 1). Our work provides a solid basis for the prediction of the concentrations of these trace elements and may also give an indication of the sites and mechanisms with which they may be involved in RA, such as impaired T cell mediated immunity¹⁷ in synovial tissue and hyperactive neutrophil function⁴ in synovial fluid.

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