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

Satish Kumar Taneja, Reshu Mandal

Department of Zoology, Panjab University, Chandigarh, India

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 serum C-reactive protein (CRP), erythrocyte sedimentation rats (ESR) vs. serum Cu, Mg and Mn, and negative with serum Zn {r= 0.973**, 0.978** (Cu), 0.978**, 0.979** (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.

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.[1] 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,[2,3] the antioxidant defense system[4] and the immune system.[5,6] 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.[7,8] 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.[4,9-13]

Previous studies have reported low dietary intakes of Zn, Cu and Mg in RA patients[14] resulting in decreased activities of antioxidant enzymes including Cu-Zn superoxide dismutase, glutathione peroxidase and catalase.[15-17] Therefore, in many clinical trials, therapeutic supplementation of Zn or Cu in RA patients has been investigated which yielded contradictory results.[7,18,19]

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[20] or from vegetables and meat food stuffs (40 mg/kg or more in above ground vegetables and 120 mg/kg or above in underground vegetables) as in Chandigarh, India.[21,22] Excessive intake of Zn has been reported to induce Cu, Mg, Mn, Fe and Se deficiencies due to their antagonistic effects[19,23,24] and also it induces inflammatory response through cytokine production by polymorpho-nuclear cells which is a consistent feature in RA.[10,25] 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).

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). Regarding their eating habits, most were vegetarians and only a few were occasional meat eaters consuming not more than 200 g of fresh chicken or goat meat/week. All of them were consuming 250 mL of day either full fat milk or skimmed milk. Intake of meat in occasional meat eaters was not significant enough; therefore, the data of vegetarians and occasional meat eaters were pooled for the present investigations.

Selection criteria and disease diagnosis (clinical tests)

In the present study, RA patients were selected on the basis of at least three of the following: morning stiffness for more than 45 min, five swollen joints, five tender joints and ESR more than 45 mm/1h.[26] The characteristics of RA patients were included in Table 1 (clini-

Correspondence: Satish Kumar Taneja, Department of Zoology, Panjab University Chandigarh 160014, India
E-mail: sktaneja@gmail.com
sktaneja@hotmail.com

Key words: Zn, Cu, Mg, Mn, rheumatoid arthritis.

Acknowledgments: thanks are due to Prof. M. Jain for laboratory facilities. Financial assistance of Panjab University, Chandigarh to R. Mandal is gratefully acknowledged. Thanks are due to Dr. H.C. Gupta from Orthopedeatric Clinic, Sector-16-D, Chandigarh (Orthopediatric and General Surgeon, Former Head of Department of Surgery and Orthopedics, General Hospital, Sector-16-D, 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.

Received for publication: 10 March 2009
Revision received: 9 June 2009.
Accepted for publication: 3 July 2009.

Rheumatology Reports 2009; 1:e5
doi:10.4081/rr.2009.e5

cal tests). The blood serum samples of RA patients and healthy subjects (hemolyzed blood samples excluded) were collected from the clinic and were stored at -20°C for further analysis of minerals by standard laboratory methods in the Department of Zoology, Panjab University, Chandigarh.

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.[27] Subsequently, the hair samples were soaked in acetone to remove external contamination, then in alcohol, rinsed five times with deionized water, dried in an oven and stored in desiccators for further analysis.

Analysis of minerals in the blood serum, urine and hair of healthy subjects and RA patients using atomic absorption spectrophotometer

Prior to sub-sampling for analysis, the samples of blood serum and urine were shaken vigorously for one min to ensure a homogeneous suspension. Samples of blood serum (1 mL), urine (1 mL) and hair (50 mg) were digested separately in 3:1 nitric acid and perchloric acid in triplicates. 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).

Table 1. Characteristics of RA patients in Group-RA (duration of RA disease 2.53±0.25 years) and their age-gender matched (females) healthy subjects in control Group-A including mean age (years), body weights (gm), RA-factor, serum C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), hemoglobin (Hb) and HDL-c levels in the population of Chandigarh, India. [Values are mean ± S.E.M. (n = 100)].

Parameters	Group-A (Control)	Group-RA	Reference values
Mean age	49.4±0.23	49.30±0.23	-
B. wt.	62.25±2.52	60.52±2.12	-
RA-factor	Negative	Positive	Negative (less than <1:20)
CRP	1.3±0.18	22.8±0.27[a]	(0-6 mg/L)
ESR	17.3±0.51	51.3±1.11[a]	(10-20 mm/1hr)
Hb	13.60±0.19	9.22±0.27[a]	(12-16 gm/dL)
HDL-c	48.65±0.92	33.41±0.46[a]	(45-65 mg/dL)

p-values: [a]<0.001 (values of group-RA were compared with control group-A).

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, 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, $p<0.001$, 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** (Mn) and -0.845** (Zn), **$p<0.0001$; 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 = 0.833** (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.[28,29]

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.[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[11,12] 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.[10] It is believed that the accumulation of Zn in Zn-containing protein metallothionein, a labile source of Zn for its distribution to inflamed joints[30] and many other organs during inflammation,[31] requires more Zn to activate yet unidentified metalloenzymes that are essential during the stress conditions leading to hypozincemia in RA.[10,32-34] In spite of higher intake of Zn from the diet by RA patients in this region, the observed hypozincemia does not support the concept *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.[10-12] The present results, therefore, do not support Zn deficiency as 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)[21] for the last two decades and reaffirm the finding of Craing *et al.*[35] who suggested that RA patients were not Zn deficient. Further, Zn supplementation trials in RA patients suggested the contradictory results on Zn status.[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.[36,37]

The hypercupremia evaluated in RA patients was reported to be associated with inflammatory response resulting from increased oxidative stress[38] as evident by the positive correlations between serum Cu levels and inflammatory markers serum CRP and ESR in RA patients in the present study.[39,40] The cytokines have been reported to enhance the release of Cu thioneins during the oxidative burst of polymorphonuclear cells that led to hypercupremia in RA patients.[25] The hypercupreuria that develops was suggested to be the outcome of hypercupremia, a constant feature in RA

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)].

Parameters	Group-A (Control)	Group-RA
Serum Cu*	0.047±0.01	1.740±0.02[a]
Serum Zn*	0.338±0.01	0.296±0.01[c]
Serum Mg*	0.390±0.01	1.700±0.01[a]
Serum Mn*	2.230±0.07	2.880±0.02[a]
Urine Cu*	3.980±0.18	8.000±0.27[a]
Urine Zn*	0.241±0.01	0.329±0.06[c]
Urine Mg*	0.590±0.05	1.790±0.06[a]
Urine Mn*	1.140±0.20	1.450±0.07[b]
Hair Cu[§]	186.84±0.42	104.28±2.07[a]
Hair Zn[§]	47.12±0.36	33.12±0.83[a]
Hair Mg[§]	32.28±0.21	17.96±0.22[a]
Hair Mn[§]	97.12±5.65	73.88±0.94[a]

*mg/dL; [§]mg/g of tissues; *p*: [a]<0.001, [b]<0.01; [c]<0.05 (values of group-RA were compared with control group-A).

correlated positively with CRP and ESR in RA.[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 hypercupeuria, 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.[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.[42] The hypercupremia further leads to a decrease in HDL-c in RA patients resulting from Cu deficiency.[43-45] This Cu-deficiency has been reported to weaken immune system function resulting in increased infections causing increased ESR in RA patients.[46] Low HDL-c in RA further leads to inactivation of the immune system and increases the risk for inflammation in RA patients.[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.[18,48-52] 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.[53-58]

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[32] 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.[53,59]

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

1. Wolfe F, Mitchell DM, Sibley JT, et al. The mortality of rheumatoid arthritis. Arthritis Rheum 1994;37:481-94.
2. Lappalainen R, Kunnttila M, Lammi S, et al. Zn and Cu content in human cancellous bone. Acta Orthop Scand 1982;53:51-5.
3. Starcher BC, Hill CH, Madaras JG. Effect of zinc deficiency on bone collagenase and collagen turnover. J Nutr 1980;110:2095-102.

4. Milanino R, Frigo A, Bambara LM, et al. Copper and zinc status in rheumatoid arthritis: studies of plasma, erythrocytes and urine, and their relationship with disease activity markers and pharmacological treatment. Clin Exp Rheumatol 1993a;11: 271-81.
5. Fraker PJ, Jardieu P, Cook J. Zinc deficiency and immune function. Arch Dermatol 1987;123:1699-701.
6. Stabel JR, Spears JW. Effect of copper on immune function and disease resistance. Adv Exp Med Biol 1989;258:243-52.
7. Simkin PA. Oral zinc sulphate in rheumatoid arthritis. Lancet 1976;2:539-42.
8. Rainsford KD. Environment ion perturbations, especially as they affect copper status, are a factor in the etiology of arthritis conditions: an hypothesis. In: Sorenson JRJ, editor. Inflammatory disease and copper. Clifton, NJ, Humana Press; 1982. pp. 137-43.
9. Honkanen VE, Pelkonen P, Mussalo-Rauhamaa H, et al. Serum trace elements in juvenile chronic arthritis. Clin Rheumatol 1989;8:64-70.
10. Zoli A, Altomonte L, Caricchio R, et al. Serum Zinc and Copper in active rheumatoid arthritis: correlation with interleukin 1, and tumor Necrosis factor. Clin Rheumatol 1998;17:378-82.
11. Honkanen VE, Konttinen YT, Sorsa T, et al. Serum zinc, cooper and selenium in rheumatoid arthritis. J Trace Elem Electrolytes Health Dis 1988;5:261-3.
12. Honkanen VE, Lamberg-Allardt CH, Vesterinen MK, et al. Plasma zinc and copper concentrations in rheumatoid arthritis: influence of dietary factors and disease activity. Am J Clin Nutr 1991;54: 1082-6.
13. Milanino R, Marrella M, Gasperini R, et al. Copper and Zinc body levels in inflammation: an overview of the data obtained from animal and human studies. Agents and Actions 1993b;39:195-209.
14. Kremer JM, Bigaouette J. Nutrient intake of patients with rheumatoid arthritis is deficient in pyriodoxine, zinc, copper and magnesium. J Rheumatol 1996;23:990-4.
15. Hassan HQ, Hadi RA, Al-Rawi ZS, et al. The glutathione defense system in the pathogenesis of rheumatoid arthritis. J Appl Toxicol 2001;21:69-73.
16. Kiziltune A, Cogalgil S, Cerrahoglu L. Carnithine and anti-oxidants levels in patients with rheumatoid arthritis. Scand J Rheumatol 1998;27:441-5.
17. Kerimova AA, Atalay M, Yusifov EY, et al. Antioxidant enzymes: possible mechanism of gold compound treatment in rheumatoid arthritis. Pathophysiol 2000;7:209-13.
18. Cimmino MA, Mazzucotelli A, Rovetta G, et al. The controversy over zinc sulphate efficacy in rheumatoid and psoriatiric arthritis. Scand J Rheumatol 1992;13:191-2.
19. Aaseth J, Haugen M, Forre OC. Rheumatoid arthritis and metal components – perspective on the role of oxygen radical detoxification. Analyst 1998;123:3-6.
20. Arsenault JE, Brown KH. Zinc intake of US preschool children exceeds new dietary reference intakes. Am J Clin Nutr 2003; 78: 1011-7.
21. Ram B, Garg SP, Matharu SS. Effect of contaminants in wastewater on soil and vegetables - a case study. Panjab Pollution Control Board 2005.
22. Taneja SK, Mandal R. Mineral factors controlling essential hypertension- a study in the Chandigarh, India population. Biol Trace Elem Res 2007;120:61-73.
23. Akahori A, Gabryelak T, Jozwiak Z, Gondko R. Zinc-induced damage to carp (Cyrinus Carpio L.) erythrocytes in vitro. Biochem Mol Biol Int 1999;47:89-98.
24. Yadrick MK, Kenney MA, Winterfeldt EA. Iron, copper, and zinc status: response to supplementations with zinc or zinc and iron in adult females. Am J Clin Nutr 1989; 49:145-50.
25. Driessen C, Hirv K, Kinchner H, Rink L. Zinc regulate cytokine induction by superantigens and lipopolysaccharide. Immunology 1995;84:272-7.
26. Wanchu A, Deodhar SD, Sharma M, et al. Elevated levels of antiproteus antibodies in patients with active rheumatoid arthritis. Indian J Med Res 1997;105: 39-42.
27. Gammelgaard B, Peters K, Menno T. Reference values for the nickel concentrations in human fingernails. J Trace Elem Electrolyte Health Dis 1991;5:121-3.
28. Akyol O, Kavutcu M, Canpolat O, et al. A study on the relationships between some of the trace elements levels of hair, nail, serum, and urine in healthy subjects. Turk J Med Res 1992;11:75-81.
29. Magolova T, Bella V, Brtkova A, et al. Copper, zinc and superoxide dismutase in precancerous, benign diseases and gastric, colorectal and breast cancer. Neoplasma 1999;46:100-4.
30. Akcil E, Seckin B, Ergun A, Hatungil R. The effects of ultrasound on synovial fluid zinc level in patients with rheumatoid arthritis. Trace Elements and Electrolytes 2006;23: 55-9.
31. Abel J, de Ruiter N. Inhibition of hydroxyl-radical-generated DNA degradation by metallothionein. Toxicol Lett 1989;47:191-6.
32. Taysi S, Gulcin I, Sari RA, et al. Trace elements and disease activity score in patients with rheumatoid arthritis. The Pain Clinic 2004;15:435-9.
33. Clegg MS, Ferrell F, Keen CL. Hypertension-induced alterations in copper and zinc metabolism in dahl rats. Hypertension 1987;9:624-8.
34. Akintola DF, Sampson B, Burrin JB, et al. Changes in plasma metallothionein-1, Interleukin-6, and C - reactive protein in patients after elective surgery. Clin Chem 1997;43:845-7.
35. Craing GM, Evans SJ, Brayshaw BJ. An inverse relationship between serum zinc and C-reactive protein levels in acutely ill elderly hospital patients. Postgrad Med J 1990;66:1025-8.
36. Boosalis MG, Solem LD, McCall JT, et al. Serum zinc response in thermal injury. Am J Clin Nutr 1988;7:69-76.
37. Gambino R. C-reactive protein-undervalued, underutilized. Clin Chem 1997;43:2 017-8.
38. Ford ES. Serum copper concentrations and coronary heart disease among US adults. Am J Epidemiol 2000; 151:1182-8.
39. Liuzzo G, Biasucci LM, Gallimore JR, et al. The prognostic value of C-reactive protein and serum amyloid a protein in severe unstable angina. N Engl J Med 1994;331: 417-24.
40. Salonen JT, Salonen R, Korpela H, et al. Serum copper and the risk of acute myocardial infarction: a prospective population study in men in eastern Finland. Am J Epidemiol 1991;134:268-76.
41. Aaseth J, Munthe E, Forre O, Steinnes E. Trace elements in serum and urine of patients with rheumatoid arthritis. Scand J Rheumatoid 1978;7: 237-40.
42. DiSilvestro RA, Marten J, Skehan M. Effects of copper supplementation on ceruloplasmin and copper-zinc superoxide dismutase in free-living rheumatoid arthritis patients. Am J Clin Nutr 1992;11:177-80.
43. Kromhout D, Wibowo AAE, Herber RFM, et al. Trace metals and coronary heart disease risk indicators in 152 elderly men (the Zutphen Study). Am J Epidemiol 1985;122:378-85.
44. Lefevre M, Keen CL, Lonnerdal B, et al. Copper deficiency-induced hypercholesterolemia: effects on HDL sub-fractions and hepatic lipoprotein receptor activity in the rat. J Nutr 1986;116:1735-46.
45. Lee CC, Koo SI. Effect of copper deficiency on the composition of three high-density lipoprotein subclasses as separated by heparin-affinity chromatography. Biochim Biophys Acta 1988;963:278-87.
46. Bonham M, O'Connor JM, Hannigan BM, Strain JJ. The immune system as a physiological indicator of marginal copper status. Br J Nutr 2002;87:393-403.
47. Geogiadis AN, Papavasiliou EC, Lourinda ES, et al. Atherogenic lipid profile is a feature characteristic of patients with early rheumatoid arthritis: effect of early treatment – a prospective, controlled study. Arthritis Res Ther 2006;8:R82.

48. Broun ER, Greist A, Tricot G, Hoffman R. Excessive zinc ingestion. A reversible cause of sideroblastic anemia and bone marrow depression. JAMA 1990;264:1441-3.
49. Kumar N, Gross JB, Ahlskog JE. Myelopathy due to copper deficiency. Neurology 2003;61:273-4.
50. Sugiura T, Goto K, Ito K, et al. Chronic zinc toxicity in an infant who received zinc therapy for atopic dermatitis. Acta Paediatr 2005;94:1333-5.
51. Arredondo M, Martinez R, Nunez MT, et al. Inhibition of iron and copper uptake by iron, copper and zinc. Biol Res 2006;39:95-102.
52. Wapnir RA and Balkman C. Inhibition of copper absorption by zinc. Effect of histidine. Biol Trace Elem Res 1991;29:193-202.
53. Beisel WR. Single nutrient and immunity. Am J Clin Nutr 1982;35:417-68.
54. Snedeker SM, Greger JL. Metabolism of zinc, copper and iron as affected by dietary protein, cystein and histidine. J Nutr 1983;113:644-52.
55. Whittaker P. Iron and zinc interactions in humans. Am J Clin Nutr 1998;68:442S-46S.
56. Zago MP, Oteiza PI. The antioxidant properties of zinc: interactions with iron and antioxidants. Free Rad Biol Med 2001;31:266-74.
57. Chen H, Huang G, Su T, Gao H, et al. Decreased hephaestin activity in the intestine of copper-deficient mice causes systemic iron deficiency. J Nutr 2006; 136:1236-41.
58. Uriu-Adams JY, Keen CL. Copper, oxidative stress and human health. Mol Aspects Med 2005;26:268-98.
59. Vural H, Uzun K, Uz U, et al. Concentrations of copper, zinc and various elements in serum of patients with bronchial asthma. J Trace Elem Med Biol 2000;14:88-91.