

Report

Serum zinc and copper status in Iranian patients with pemphigus vulgaris

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Abstract

Background The role of nutritional factors including trace elements has been reported in the pathogenesis of many autoimmune diseases.

Objective Regarding the relatively high prevalence of pemphigus vulgaris in Iran, we investigated the serum levels of zinc and copper as two important trace elements, together with the oxidative stress status in patients with pemphigus vulgaris.

Materials and methods This case-control study was performed on 25 patients with newly diagnosed pemphigus vulgaris and 25 age- and sex-matched healthy control subjects. Serum concentrations of zinc, copper, ceruloplasmin as well as copper/zinc ratio were determined for each subject. Oxidative stress was also measured using a novel assay of peroxidant-antioxidant balance (PAB).

Results Mean serum concentrations of zinc and copper as well as copper/zinc ratio were significantly lower in patients (mean age: 47.2 ± 16.2 years; male/female: 14/11) compared with the controls (mean age: 47.3 ± 12.8 years; male/female: 14/11; $P < 0.001$). In contrast, PAB values were significantly elevated in patients compared with controls ($P < 0.01$). No significant difference in serum ceruloplasmin concentrations was observed between the groups ($P > 0.05$).

Conclusion Our findings indicate that low serum zinc and copper and increased oxidative stress may be associated with pemphigus vulgaris.

Introduction

In dermatology, some disorders are caused by nutritional factors with different mechanisms, i.e. by immune mechanism in dermatitis herpetiformis, or deficiency of nutrients such as vitamin C in scurvy. It is also well documented that nutritional status can significantly affect the function of the immune system, autoimmunity, and resistance to infections. It has been proven that deficiency of zinc and copper as two essential trace elements plays an important role in autoimmunity mechanisms.¹⁻³

Pemphigus vulgaris is an autoimmune blistering disease that affects the skin and mucous membranes and is mediated by circulating autoantibodies directed against keratinocyte cell surfaces.⁴ The role of nutritional factors in pemphigus vulgaris has been proven by epidemiological, clinical, and laboratory data.¹ Although there are some previous reports on the role of trace elements in auto-

immune diseases, they are few in regard to pemphigus vulgaris. Therefore, concerning the high prevalence of pemphigus vulgaris in Iran in comparison to the Western countries^{5,6} and also the high prevalence of zinc deficiency,⁷⁻¹⁰ we aimed to determine the serum levels of zinc, copper, and ceruloplasmin (which contains an active form of copper), as well as copper/zinc ratio and peroxidant-antioxidant balance (PAB) in Iranian patients with pemphigus vulgaris.

Materials and methods**Subjects**

This case-control study was performed on new cases of patients with pemphigus vulgaris aged 20–80 years who were recruited from the Ghaem and Imam Reza hospitals (Mashhad, Iran) between January 2007 and June 2008. Patients who had other autoimmune diseases or were taking zinc sulfate or other

supplements as well as old cases of pemphigus vulgaris (older than one year) were excluded, and finally 25 eligible patients entered the study. The diagnosis of pemphigus vulgaris was confirmed by clinical, histological, and direct immunofluorescent findings. Twenty-five age- and sex-matched healthy subjects without any drug consumptions who referred to Imam Reza paraclinic for check-up were also recruited as the control group. Serum concentrations of zinc, copper, and ceruloplasmin as well as copper/zinc ratio and PAB were determined for all participants.

The study protocol was approved by Mashhad University of Medical Sciences (MUMS) Ethics Committee, and written informed consent was obtained from each participant.

Serum trace element analysis

Copper and zinc in serum were measured by flame atomic absorption spectrometry (model 3030; Perkin Elmer, Norwalk, CT, USA) as previously described.^{11,12}

Ceruloplasmin measurement

Serum ceruloplasmin concentration was determined using a radial immunodiffusion kit (BINDARID™; Binding Site, UK).

PAB assay

To assess the oxidative stress level, a modified PAB assay was applied based on a previously described method.¹³

Statistical analysis

Statistical analysis was performed using SPSS software (version 11.5, SPSS Inc., Chicago, IL, USA). Data were expressed as mean \pm SD or mean \pm SEM. Between-group comparisons were made using independent *t*-test (for numerical variables) or Chi-square test (for categorical variables). A two-tailed *P*-value of <0.05 was considered statistically significant.

Results

There was no significant difference in age and gender between patients and controls ($P > 0.05$; Table 1). Like-

Table 1 Demographic characteristics and anthropometric parameters of patient and control groups

| | Patients | Controls | <i>P</i> -value |
|--------------------------|------------------|-----------------|-----------------|
| Age (years) | 47.2 \pm 16.2 | 47.3 \pm 12.8 | >0.05 |
| Female (%) | 11 (44) | 11 (44) | >0.05 |
| BMI (kg/m ²) | 25.0 \pm 4.9 | 24.4 \pm 3.8 | >0.05 |
| Waist (cm) | 88.5 \pm 18.3 | 90.3 \pm 10.6 | >0.05 |
| Height (cm) | 163.3 \pm 10.7 | 163.3 \pm 7.9 | >0.05 |
| Weight (kg) | 66.9 \pm 13.6 | 65.4 \pm 12.2 | >0.05 |

Values are expressed as mean \pm SD or number (%). Between-groups comparisons were made using independent *t*-test or Chi-square test.

BMI, body mass index.

wise, the groups did not significantly differ in their anthropometric parameters, including weight, height, body mass index, and waist circumference ($P > 0.05$; Table 1).

Overall the patients had significantly lower serum concentrations of zinc and copper compared with the corresponding values for the control group ($P < 0.001$; Table 2). In the same manner, the copper/zinc ratio was also significantly lower in patients compared with controls ($P < 0.001$; Table 2). The prevalence of zinc and copper deficiency (defined as serum levels lower than the reference range) was higher in patients compared with control subjects ($P = 0.05$ and $P < 0.001$ for zinc and copper, respectively). However, mean serum ceruloplasmin value did not differ significantly between the groups ($P > 0.05$; Table 2). With regard to the PAB values, a significant elevation was observed in patients compared with the controls, which is a sign of heightened state of oxidative stress in these patients ($P < 0.01$).

Discussion

The incidence of pemphigus vulgaris is about 10/100,000 per year in Iran, which is higher than some other countries, such as Finland (0.76/1,000,000/year), Germany, and France (about 1/1,000,000/year). The mean age of disease onset is between 40 and 60 years. The prevalence of disease between males and females is similar in many areas like Iran.¹⁴ Formation of IgG autoantibodies against the cell surface of keratinocytes is considered to be the main pathomechanism of the disease. Immunological evidences indicate that antigens are desmogleins, as inactivation of these proteins in other ways such as by toxins can

Table 2 Comparison of serum trace element status and PAB values between patient and control groups

| | Patients | Controls | <i>P</i> -value |
|--------------------------------|---------------------|--------------------|-----------------|
| Zn (μ g/l) | 914.2 \pm 311.9** | 1207.2 \pm 173.8 | <0.01 |
| Cu (μ g/l) | 769.6 \pm 240.7** | 1642.8 \pm 334.4 | <0.01 |
| Cu/Zn | 1.2 \pm 1.6** | 1.4 \pm 0.3 | <0.01 |
| Cp (mg/l) | 277.4 \pm 84.7 | 244.9 \pm 86.9 | >0.05 |
| PAB (AU) | 118.9 \pm 39.3* | 79.1 \pm 29.7 | <0.05 |
| Zn deficiency ^a (%) | 20 | 0 | 0.05 |
| Cu deficiency ^b (%) | 48 | 0 | <0.001 |

Values are expressed as mean \pm SEM. Between-group comparisons were made using independent *t*-test.

* $P < 0.01$; ** $P < 0.001$.

^aDefined as serum Zn < 699.3 μ g/l in both males and females.

^bDefined as serum Cu < 700 μ g/l in males and <800 μ g/l in females.

AU, absorbance unit; Cp, caeruloplasmin; Cu, copper; PAB, peroxidant-antioxidant balance; Zn, zinc.

also lead to acantholysis.^{14,15} Previously, it was reported that blocking of intracellular signal pathways can prevent antibody-mediated acantholysis.¹⁴ Besides, the role of the plasminogen–plasmin system has also been shown in acantholysis, as in a previous study on experimental models, protease inhibitors, and plasminogen-activating inhibitors blocked acantholysis.¹⁶

Serum zinc and copper have been reported to be associated with the immune response, inflammation, and oxidative stress in the human body.^{17–22} Copper/zinc ratio is also considered as a useful marker of malnutrition, in addition to other classic anthropometric and biological nutritional parameters.^{22,23} About 20% of body total zinc is located in the skin. It has been reported that zinc has a role in protein and nucleic acid synthesis and also the function of T-lymphocytes.²⁴ Therefore, zinc deficiency – which is prevalent in Iran – leads to the thymus atrophy and impairment of cell and antibody-mediated immunity.²⁵ In previous studies, decreased concentrations of zinc were reported in autoimmune diseases, such as diabetes mellitus and asthma, which is concordant with our findings in the present study.^{26–28}

Copper is also an important constituent of metalloenzymes, and its role in oxidation–reduction systems and against free radicals has been proven. Copper deficiency leads to a decrease in antibodies, thymus weight, and T-lymphocytes, as well as increased oxidative injuries.²⁹ Unlike zinc, the results of studies concerning copper status in autoimmune diseases (asthma and diabetes mellitus) indicated higher levels of this trace element in patients.^{26–28} In our study, the ceruloplasmin level in patients was higher than that of controls, though this difference did not reach statistical significance. In a previous study, serum ceruloplasmin levels were reported to be higher in patients with asthma and type 2 diabetes compared with the controls.^{26,30}

With regard to pemphigus vulgaris, it has been shown that copper levels of mononuclear blood cell chromatin in four patients with pemphigus vulgaris were significantly lower than values of control subjects. However, although zinc levels were also lower in patients with pemphigus vulgaris, the difference did not reach statistical significance.³¹

It should be noted that poor nutrition (due to mouth sores, which may lead to painful eating) is also a possible cause for the lower serum levels of zinc and copper in individuals with pemphigus vulgaris. On the other hand, penicillamine – which is a drug with copper chelating activity – has been found to be a well-known inducer of pemphigus,^{32,33} implying that copper deficiency may also be involved in the induction of pemphigus vulgaris.

For the evaluation of oxidative stress, we applied a recently modified PAB assay, which is a simple, rapid, and inexpensive measure of oxidative status.¹³ The results

of the PAB assay indicated a significant increase in the extent of oxidative stress in patients. Our results are consistent with a previous study in which lower antioxidant status was reported in pemphigus vulgaris.³⁴ Furthermore, similar results have been reported for other autoimmune diseases, such as rheumatoid arthritis³⁵ and systemic lupus erythematosus,³⁶ which may support the role of oxidative stress in the pathogenesis of these diseases.³⁷ Regarding the well-known protective roles of zinc^{38–40} and copper⁴¹ against oxidative stress, the raised PAB values in patients could be attributed to decreased serum levels of these important trace elements.

In summary, we reported decreased serum levels of zinc and copper and increased state of oxidative stress in patients with pemphigus vulgaris. The casual relationship between pemphigus vulgaris and low serum zinc and copper status needs to be determined by future studies. However, regarding the very limited data on the status of these trace elements in pemphigus vulgaris, the present study may generate hypothesis for future studies to investigate the possible mechanisms behind lower serum zinc and copper in this disease and whether these changes could be applied as risk factors for pemphigus vulgaris.

Acknowledgments

This research project has been financially supported by the Research Council of the Mashhad University of Medical Sciences. We would also like to thank all the study participants who devoted their time to this study.

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