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CLINICAL STUDIES

Prevalence of zinc deficiency and immune response in short-term hemodialysis

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Abstract

Zinc is an essential trace element for many biological functions, including immune functions. The mechanism by which zinc may affect the immune system is certainly multifaceted, due to zinc's widespread action on different enzymes, peptides, transcriptional factors and cytokines involved in the various physiological steps of immune development and reactivity. In this study, prevalence of zinc deficiency and alteration in complement system, immunoglobulins and T cell subsets depending on zinc levels were analyzed in short-term hemodialysis patients and compared with healthy controls. Plasma zinc levels were measured by flame atomic absorption spectrometry. Serum levels of complement C_3 and C_4, immunoglobulins G (IgG), M (IgM), and A (IgA), and prealbumin were measured by nephelometry depending on antigen-antibody reactions. Percentages of CD_4^+ and CD_8^+ were calculated using a flow cytometer. Statistically significant decreased zinc levels, especially in the age group $\geqslant$40 years, and increased C_4, IgA, IgM, IgG and CD_4^+ levels were observed in hemodialysis patients. The prevalence of hypozincemia in hemodialysis patients was found to be 40%. A higher CD_4^+/CD_8^+ ratio was also obtained in patients. We conclude that patients on maintenance hemodialysis for a short time exhibit zinc deficiency and disturbed immune response.

Keywords: Hemodialysis; Immunoglobulins; Complement system; T cells; Zinc

Introduction

Zinc is one of the most important trace elements in the body. Being a catalytic component of more than 300 enzymes, zinc has a structural and biological role in many proteins, peptides, hormones, transcriptional and growth factors and cytokines, involved in the various steps of immune development and reactivity [1,2]. Its participation in the synthesis of nucleic acids and proteins [3], and possibly in the structure and function of biomembranes seems to account for its role in immunity [4]. Numerous data in experimental animals support the existence of a crucial role played by zinc [5], and the essentiality of zinc for immune functions has been established through many studies [6–10].

It is well known that trace element disturbances can be a result of uremia, and trace element metabolism can be affected by reduced renal function, alteration of the metabolic balance, special diet, medication and dialysis treatment in renal insufficiency. Most element disturbances are shared by dialyzed and nondialyzed uremic patients. However, the severity of the disturbance is

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greater in dialyzed patients [11–14]. Various trace element abnormalities in long-term hemodialysis patients have been reported [11,15–16].

There is a general agreement on clinical ground that patients on maintenance dialysis have an impaired immune response [17–19]. A considerable body of evidence indicates that various components of the immune system are impaired in uremic patients. Some studies of humoral immunity in uremia have reported a normal response to antigenic stimuli [20–22], while others have found a reduced antibody response [23,24]. The complement system plays an important role in nonspecific host resistance and complement components are involved in the regulation of specific humoral and cellular immunity [25]. Subnormal concentrations of serum immunoglobulins and complement components have been reported in uremic patients [26].

We aimed to determine alterations in immunological parameters; immunoglobulins, complements and T cell subsets depending on plasma zinc levels, and to analyze the prevalence of the deficiency of these parameters in patients on short-term hemodialysis compared with healthy subjects.

Materials and methods

Patients

In total, 72 patients with chronic renal disease (41 male and 31 female patients, mean age 48.21 ± 13.33 (range 22–72) years), and 52 healthy control subjects (27 male and 25 female subjects, mean age 36.58 ± 12.06 (range 20–72) years) were included in this study. Subjects with diseases associated with immune disorders or who were taking medications known to alter immune function were excluded from the control group. All patients were undergoing routine hemodialysis three times/week on AK 90 and AK 100 machines using cuphrophane membranes and standard dialysate at the Başkent University Hospital. The mean duration of hemodialysis was 48–50 months. All patients were on a 1.2 g protein/kg per day and 35 kcal/kg per day caloric diet, and did not receive drugs known to influence the immune response. None of the patients and controls had acute or chronic infections at the beginning of the study. Oral consent was obtained from the subjects before the study.

Samples and parameters measured

Fasting predialysis blood samples were collected and centrifuged at 3800 rpm within 2 h. Samples were stored at −20 °C until analysis. For complements, immunoglobulins, albumin, prealbumin and total protein determination, blood samples were collected in anticoagulant free vacutainer tubes. For zinc determination, blood samples were collected in trace element free vacutainer tubes containing K_2EDTA. For CD_4^+ and CD_8^+ determination, blood samples were collected in vacutainer tubes with K_2EDTA.

Plasma zinc levels were measured with a Shimadzu AA 1060 flame atomic absorption spectrometer. For the analysis, one volume plasma was mixed with four volumes nitric acid (0.1%, v/v), and the sample then applied to flame atomic absorption spectrometry [27]. All samples were run in triplicates. $Zn(NO_3)_2$ in 0.1 mol/l HNO_3 was used as standard. BioRad zinc standards (BioRad, USA) were used as reference samples.

Serum levels of complement C_3 and C_4, immunoglobulin G (IgG), M (IgM), and A (IgA), and prealbumin were measured by nephelometry depending on antigen–antibody reactions (Beckman 360 Specific Protein Analyser). Human C_3 and mammalian C_4, human IgG, IgA, IgM and prealbumin antibodies in 0.1% (w/v) sodium azide were used for these reactions.

Anti-CD_4-FITC (fluorescein-isothyocyanate) and anti CD_8-PE (phycoerytrin) were used to determine CD_4^+ and CD_8^+. Percentages of CD_4^+ and CD_8^+ were calculated using a flow cytometer (Coulter EPIC XL-MCL, USA).

Total protein and albumin levels were determined colorimetrically with commercial kits (Stanbio, USA).

Statistics

SPSS 8.0 software was used for statistical analysis. Data were given as mean ± SD. Mann–Whitney U test and Spearman's rho correlation analysis were used. The level of statistical significance was set at $p < 0.05$.

Results

Table 1 shows the concentrations of plasma zinc, immunoglobulins, T cell subset and complement system parameters in patients and controls. The mean value of plasma zinc was lower ($p < 0.0001$) and mean values of C_4, IgA, IgM, IgG and CD_4^+ were higher in patients than in controls. When compared to controls, a higher CD_4^+/CD_8^+ ratio was observed in hemodialysis patients ($p < 0.0001$). In total, 29 patients presented a zinc concentration below the lower limit of the normal range (10.7–18.4 μmol/l). The mean values of the other determined parameters were within the normal range in both groups (Table 1). Hypozincemia prevalence in hemodialysis patients was 40%. Hypozincemic hemodialysed patients had significantly higher IgM, and lower prealbumin levels than patients having plasma zinc levels in the normal range ($p < 0.01$, 0.001,