Original Article

Serum zinc and selenium in children with inflammatory bowel disease: a multicenter study in Japan

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Abstract

Background: Reports of zinc and selenium deficiencies accompanying inflammatory bowel disease (IBD) mostly have originated from Western countries and concerned adult patients. Whether Japanese children with IBD have similar deficiencies remained unclear.

Aim: We aimed to elucidate differences in serum zinc and selenium concentrations in Japanese children between types of IBD.

Methods: Children under 17 years old undergoing care at 12 Japanese pediatric centers were retrospectively enrolled between November 2016 and February 2018 to 3 groups representing Crohn's disease (CD), ulcerative colitis (UC), and normal controls (NC) with irritable bowel syndrome or no illnesses. Serum zinc and selenium were measured by atomic absorption spectrophotometry. Zinc and selenium deficiencies were defined by serum concentrations.

Results: Subjects included 98 patients with CD (median age, 13 years), 118 with UC (11 years), and 43 NC (11 years). Serum zinc and selenium were significantly lower in CD (median, 64 and 12.6 μg/dL respectively) than in UC (69 and 14.6; $P<0.01$).

Conclusions: In Japanese children under 17 years old, serum zinc and selenium were significantly lower in CD than in UC or NC. Zinc and selenium should be monitored, and supplemented when deficient, in children with IBD, especially CD.

Keywords: zinc, selenium, inflammatory bowel disease, children

Introduction

Inflammatory bowel disease (IBD) refers to a group of chronic and relapsing disorders including Crohn's disease (CD), ulcerative colitis (UC), and other chronic relapsing inflammatory conditions (IBD-unclassified or IBD-U). CD tends to cause transmural inflammation and affect any part of the gastrointestinal tract in a non-continuous type. In contrast, UC is typified by mucosal inflammation and is limited to the colon [1]. Vitamin and mineral deficiencies can occur in patients with IBD as a result of intestinal mucosal inflammation and decreased oral intake [2]. Zinc, an essential micronutrient absorbed in the small intestine, serves as a cofactor for various enzymes involved in immune function, growth, and tissue repair [3]. Zinc is essential for immune cell proliferation and influences both acquired and innate immunity by acting as a coenzyme in many key reactions of the immune response, being essential for antioxidant responses and thymic hormone function [4]. Zinc deficiency increases the number of pro-inflammatory cells and promotes intestinal release of inflammatory cytokines that aggravate mucosal leakage, worsening intestinal inflammation [5]. According to single-center studies, zinc deficiency is common in pediatric patients with IBD during both remission and active phases with a prevalence of 40% in IBD and 51% in CD [2, 6]. Recently, Siva et al. [7] concluded that zinc deficiency may contribute to disease activity in adult patients with IBD. Deficiency of selenium, another micronutrient that serves as a component of glutathione peroxidase and as a scavenger of hydroperoxides, can be associated with muscle pain and weakness, cardiomyopathy, encephalopathy, lightening of hair and skin color, and anemia [8]. As a component of selenoproteins (mainly

selenoproteins S and K), selenium may down-regulate inflammatory signaling pathways involved in the pathogenesis of IBD, including inflammatory cytokine production [9]. Mostly from Western countries, zinc and selenium deficiencies have been reported in patients with IBD, including some children [2, 7, 10–12]. Whether Japanese children with IBD have zinc and selenium deficiencies has remained unclear, so we examined serum zinc and selenium status in Japanese children with IBD in a multicenter study.

Methods

Design and ethical matters

This retrospective multicenter study was designed and conducted in 2018 by 12 Japanese pediatric centers together with Nobelpharma Co., Ltd., which established a fund to support measurement of serum zinc and selenium for this study, without corporate access to information from samples or participation in data analysis. We measured zinc and selenium in residual selenium in residual serum samples stored at -30℃ following a previous investigation of serum antibodies in diagnosis of pediatric IBD in Japan[13,14]. Our study protocol complied with the ethical guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of Kurume University and its counterparts at other participating centers.

Study subjects

Subjects under 17 years old who had residual frozen serum samples from the prior investigation of

serum antibodies in diagnosis of pediatric IBD in Japan [13, 14] were retrospectively enrolled in the present study. These subjects were divided into 3 groups representing CD, UC, and normal controls (NC). The latter group included patients with irritable bowel syndrome (IBS) as well as healthy children. All patients with UC or CD underwent endoscopy for definitive diagnosis, as did some patients with IBS. Diagnosis of IBDs followed the revised Porto criteria [15]. Demographic, clinical, and laboratory features at the time of serum collection including age, gender, IBD location and activity, treatments, and routine blood test results were considered in the analysis. Age at diagnosis and duration of IBD also were noted. Sites of IBD activity were recorded according to the Paris classification [16]. To assess IBD disease activity, we used the Pediatric Crohn's Disease Activity Index (PCDAI) [17] for CD and the Pediatric Ulcerative Colitis Activity Index (PUCAI) [18] for UC. Remission, mild CD activity, and moderate to severe activity were represented by PCDAIs below 10, 10 to 30, and above 30, respectively [17]. Remission, mild UC activity, and moderate to severe activity were represented by PUCAIs below 10, 10 to 35, and over 35, respectively [18]. We examined dietary treatment with vs. without an elemental diet using Elental® [19]. Blood tests included complete blood count, serum total protein, albumin, C-reactive protein, and erythrocyte sedimentation rate.

Serum zinc and selenium assays

Serum zinc and selenium were measured by atomic absorption spectrophotometry. Based upon previous reports, [2,20] zinc and selenium deficiencies were defined as <70 μg/dL and <9.5 μg/dL, respectively. We excluded patients treated with zinc and/or selenium preparations.

Statistical analysis

Continuous variables are expressed as medians with minimum and maximum, and categorical variables as percentages. Kruskal-Wallis, Dunn's multiple comparison, Mann-Whitney *U*, Fisher's exact, and chi-squared tests were used as appropriate. To evaluate the effect of serum zinc or selenium concentrations on CD or UC after for controlling patient characteristics, laboratory values, disease activity (PCDAI or PUCAI), and treatment, univariate analysis was conducted first; then, factors with a P value below 0.1 were included in the multivariate analysis. Statistical analyses were performed and related figures were created using GraphPad Prism version 6.05 (GraphPad Software; San Diego, CA) and Stata/MP version 15.1 (StataCorp; College Station, TX). Tests were two-sided. P values below 0.05 were considered to indicate statistical significance.

Results

Study population

We enrolled 259 subjects including 98 with CD, 118 with UC, and 43 NC (18 with IBS and 25 healthy children). Demographics of enrolled subjects are shown in Table 1. Characteristics of patients with CD

or UC are shown in Table 2.

Serum zinc status in subjects with CD, UC, and NC

Serum zinc concentrations were significantly lower in CD (median, 64 μg/dL; range, 33–124) than in UC (69 μg/dL; 41–177; $P < 0.05$) or NC (77 μg/dL; 51–159; $P < 0.01$; Fig. 1A). Zinc deficiency was significantly more prevalent in CD (60.2%) than in NC (37.2%; $P<0.05$) but not than in UC (51.7%; P=0.22; Fig. 1B). In IBD (CD plus UC groups), serum zinc concentrations were significantly lower than in NC (67 μg/dL; 33–177 vs. 77μg/dL; 51–159; $P< 0.001$) or NC (15.7 μg/dL; 9.9–18.9; $P = 0.75$)

Serum selenium status in subjects with CD, UC and NC

Serum selenium concentrations were significantly lower in CD (median, 12.6 μg/dL; range, 5.5-21.9) than in UC (14.6 μg/dL; 4.7-23.1; $P<0.001$) or NC (15.7 μg/dL; 9.9-18.9; $P<0.001$; Figure 2A). Selenium deficiency was significantly more prevalent in CD (15.3%) than in UC (5.9%; $P<0.05$) or NC (0%; $P<0.01$; Figure 2B). Serum selenium concentrations also were lower in IBD than in NC (13.9 μg/dL; 4.7-23.1 vs. 15.7 μg/dL; 9.9-18.9; $P<0.01$), and selenium deficiency was more prevalent in IBD than in NC (10.2% vs. 0%; $P<0.05$). Comparing patients with IBS and to the healthy children in NC group, no significant differences were in serum selenium concentrations (15.9 μg/dL; 9.9-18.9 vs.

15.1 μg/dL; 11.6-18.9, respectively ; *P=0.60*) or prevalence of selenium deficiency (0% vs. 0%; *P=1.00*).

Analysis of factors affecting serum zinc and selenium levels in patients with CD and UC

To explore factors that might affect serum zinc and selenium concentrations in patients with CD and UC, we performed univariate followed by multivariate analyses concerning patient characteristics, blood test results, disease activity (PCDAI or PUCAI), and treatments (Supplementary Tables S1 to S4). In the multivariate analysis for zinc in CD, we found presence of a perianal lesion and serum total protein to be positively associated with zinc concentration and immunomodulator to be negatively associated (Supplementary Table S1). In the multivariate analysis for selenium in UC, disease duration and red blood cell count showed significant positive associations with selenium concentration (Supplementary Table S4). Multivariate analyses for zinc in UC and selenium in CD disclosed no significant associations (Supplementary Tables S2 and S3).

Discussion

In the first large cohort of Japanese children and adolescents with IBD to be investigated concerning serum zinc and selenium status, we found zinc deficiency to be common.

In previous reports, prevalence of zinc deficiency in otherwise healthy children ranged from 14.1% to 27.8% [21–23]. In a Korean single-center study, Han et al. [20] found prevalence of zinc deficiency in

adult patients with IBD to be 39.0%, linking age under 40 years with increased risk for this deficiency. Alkhouri et al. [2] similarly reported a 40.0% prevalence of zinc deficiency among North American pediatric patients with IBD. In our Japanese multicenter pediatric study, prevalence of zinc deficiency in pediatric patients with IBD was greater (IBD overall, 55.6%; CD, 60.2%; UC, 51.7%) than in previous reports. In the North American report, serum zinc in pediatric CD (mean, 55.4 μg/dL) was significantly lower than in UC (74.7 μg/dL) or healthy controls (75.1 μg/dL) [10]. Our study also demonstrated significantly lower serum zinc in CD (median, 64 μg/dL) than in UC (69 μg/dL) or NC (77 μg/dL). Likely factors contributing to zinc defciency in children with IBD include excessive losses of zinc from the gastrointestinal tract, poor absorption, and inadequate intake [2]. Zinc is absorbed mainly in the duodenum and upper small intestine [24]. Intake may be limited by disease activity or diet therapy [25]. We suspect that patients with CD have lower zinc than those with UC because of the small intestinal localization of CD lesions and a frequent need for dietary therapy.

Public Health Service Centers in the USA recommend daily intakes of 20 to 100 μg selenium per day in adults and 2 to 3 μg/kg/day in children [26]; the Japanese Ministry of Health, Labor and Welfare suggests 25 to 30 μg/day in adults. Typical eating habits in Japan would seem unlikely to cause selenium deficiency. As with zinc, selenium is absorbed mainly in the small intestine, and selenium deficiency has been reported in small intestinal disorders [27]. Han et al. [20] reported a 30.9% prevalence of selenium deficiency in adult patients with IBD, similarly to results obtained in other studies [11, 12, 28]. On the other hand, Sikora et al. [10] reported absence of selenium deficiency in

pediatric IBD. A general review of trace elements in IBD noted presence of selenium deficiency even during remission of IBD, and low serum selenium in IBD was consistently associated with increased UC or CD activity [9]. In our Japanese pediatric study, prevalence of selenium deficiency in pediatric patients with IBD was 10.2% (CD, 15.3%; UC, 5.9%). As previously reported, we found selenium to be significantly lower in CD than in UC and NC. We believe that more than typical numbers of our pediatric IBD subjects had selenium deficiency because most patients with IBD in this study already had received medications, and more than half of them were in a remitting phase at the time when serum samples were collected. Previous studies have reported that patients with CD who have undergone small bowel resection exceeding 200 cm are at risk of developing severe selenium deficiency [29], as are patients with CD who are maintained on enteral nutrition; further, even patients without resection or enteral nutrition may develop selenium deficiency during active phases of the disease [30]. In our Japanese pediatric study, we excluded patients with bowel resections, and we found no significant correlation in patients with CD between serum selenium concentration and elemental diet. As is true for zinc, selenium deficiency in IBD largely reflects increased losses from the gastrointestinal tract [31]. While inflammation in UC is confined to the colonic mucosa, inflammation in CD involves all layers of the small intestine, making CD more likely to be complicated by deficiency of zinc and selenium than UC. On the other hand, pediatric patients tend to have more severe and extensive UC than adults [32]. A previous study found that patients with IBD associated with serum zinc deficiency were more likely to have adverse disease-specific outcomes [7], while another investigation noted that

zinc supplementation restored more normal permeability in patients with CD in remission [33]. Based on previous reports and our present study, we recommend monitoring and supplementation when needed for zinc and selenium in pediatric IBD–particularly CD, which is more prone to cause deficiencies. We investigated possible associations in pediatric patients with CD and UC between serum zinc or selenium and various factors including patient characteristics, laboratory findings, and treatments. Multivariate analysis for zinc concentration in CD found a perianal lesion and serum total protein to be significantly positively correlated, and immunomodulator to be negatively correlated, with zinc concentrations. We suspect that serum total protein correlated positively because zinc is extensively present in serum proteins as a functionally essential cofactor, as it is in more than 10% of proteins encoded by the human genome [34]. We also believe that immunomodulator correlated negatively because of its greater frequency of use in more severely ill patients. We are unable to account for the positive correlation shown for a perianal lesion. Multivariate analysis for selenium in UC showed a significant positive correlation for both disease duration and red blood cell count. We believe that serum selenium may have a positive correlation with disease duration because the longer course of treatment before serum samples were taken gave patients suficient time to improve their nutritional status and then maintain clinical stability with effective medications and proper nutrition. However, we are unable to account for the positive correlation with the blood cell count. Multivariate analyses for zinc in UC and selenium in CD detected no significant correlations. To our knowledge, this is the first large cohort of children and adolescents with IBD in East Asia to be investigated

concerning serum zinc and selenium status. Despite favorable study size, a number of limitations are present. First, our sample included only Japanese subjects, so applicability of our findings to other Asian countries or different ethnic groups should not be assumed; similar investigations of multiple patient populations and ethnic groups are needed. Second, most patients diagnosed with any sort of IBD already were receiving one or more treatments at the time of serum collection. Third, control patients (NC) were fewer than patients with CD and UC. Fourth, we were unable to analyze details of subjects' dietary treatment. Fifth, most patients had been treated with medications before enrollment. Sixth, conditions for collection of serum samples, such as time of day and fasting vs. non-fasting, were not standardized. To address these limitations, a new large prospective controlled study is desirable. In conclusion, zinc deficiency is common in Japanese children with IBD. Serum zinc and selenium were significantly lower in Japanese children with CD than in UC or NC. Our study demonstrates a need for monitoring of zinc and selenium in children with IBD, particularly CD, with supplementation initiated when deficiencies are detected.

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Figure legends

Fig. 1. Serum zinc status in CD, UC, and NC. [A] Serum zinc concentrations were significantly lower in patients with Crohn's disease (CD; median, 64 μg/dL; range, 33-124) than in ulcerative colitis (UC; 69 μg/dL; 41-177; *P<0.05*) or normal controls (NC; 77 μg/dL; 51-159; *P<0.01*). Boxes and whiskers represent median and interquartile range. [B] Prevalence of zinc deficiency was significantly higher in CD (60.2%) than in NC (37.2%; *P<0.05*), but not significantly higher than in UC (51.7%; *P=0.22*). *, *P<0.05*; **, *P<0.01*.

Fig. 2. Serum selenium status in CD, UC, and NC. [A] Serum selenium concentrations were significantly lower in patients with Crohn's disease (CD; median, 12.6 μg/dL; range, 5.5-21.9) than in ulcerative colitis (UC ;14.6 μg/dL; 4.7-23.1; *P<0.001*) or normal controls (NC; 15.7 μg/dL; 9.9-18.9; *P<0.001*). Boxes and whiskers represent median and interquartile range. [B] Prevalence of selenium deficiency was significantly higher in CD (15.3%) than in UC (5.9%; *P<0.05*) or NC (0%; *P<0.01*). *, *P<0.05*; **, *P<0.01*; ***, *P<0.001*.

Figure 1

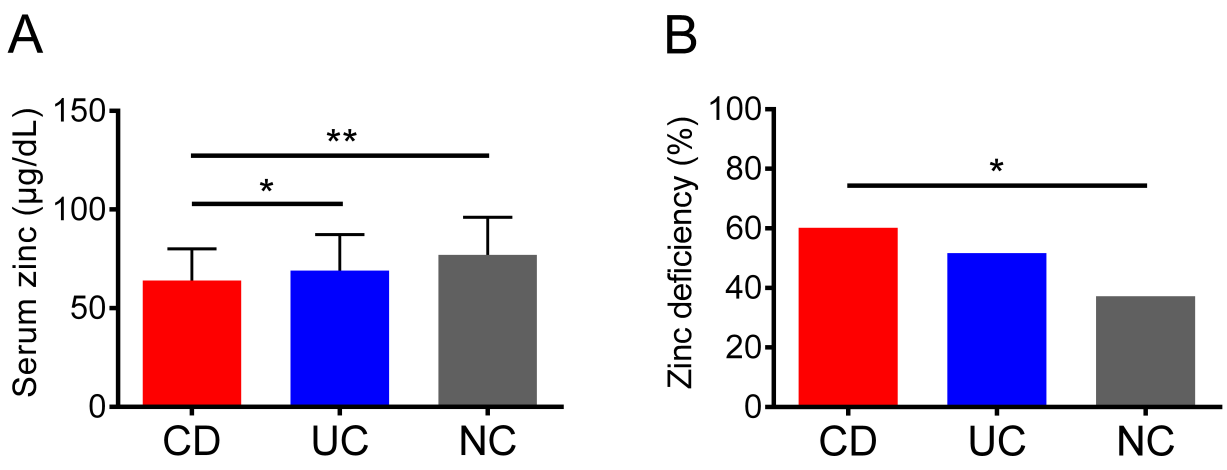
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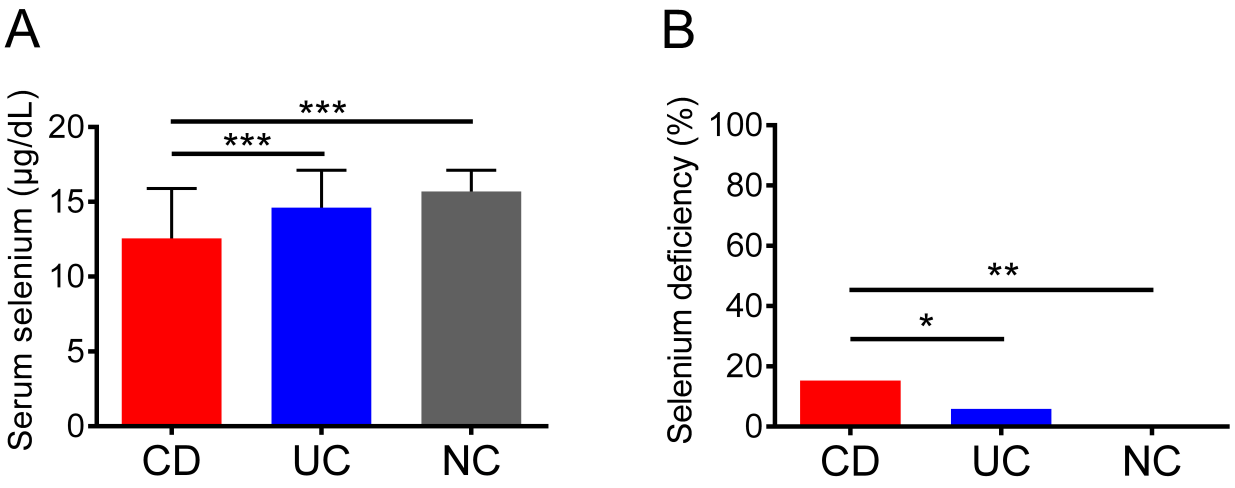
Figure 2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