Decreased serum zinc concentration in dogs with lymphocytic-plasmacytic enteritis, and its associations with disease severity and prognosis

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ABSTRACT. Human patients with inflammatory bowel disease may have poor prognosis with hypozincemia. However, there are limited data on zinc concentrations in the blood of dogs with lymphocytic-plasmacytic enteritis (LPE). The purpose of this study was to investigate the serum zinc concentration in dogs with LPE and its influence on disease severity and prognosis. Thirty-five dogs with LPE were recruited. Serum zinc concentration was measured using atomic absorption spectrometry. Hypozincemia was observed in 18/35 (51%) dogs with LPE. Serum zinc concentration was inversely correlated with histological and clinical severities. Overall survivals were significantly shorter in dogs with hypozincemia than in those without it. These findings suggest that serum zinc concentration is a useful biomarker for LPE severity and prognosis in dogs.

KEY WORDS: disease severity, dog, lymphocytic-plasmacytic enteritis, prognosis, zinc

Zinc plays pivotal roles in many aspects of cellular metabolism. These include support for catalytic activities of approximately 100 enzymes, modulation of immune function, protein synthesis, wound healing, DNA synthesis, cell division, and improvement of intestinal barrier function [7]. The gastrointestinal system is central to zinc homeostasis because it interfaces zinc exchange between organisms and the environment. Dietary zinc is primarily absorbed in the small intestine [16, 18, 24]. Hypozincemia has been observed in human patients with inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis [1, 21, 23, 27, 31]. A recent study has also shown that zinc deficiency in human patients with IBD is associated with poor clinical outcomes [27]. These necessitate close monitoring of serum zinc concentration in human patients with IBD.

Lymphocytic-plasmacytic enteritis (LPE) is often observed in dogs with chronic enteritis, including IBD. Although the detailed pathology is unclear, previous studies have reported that concentrations of serum minerals, such as calcium and magnesium, are decreased in dogs with LPE [5, 8, 15]. However, the zinc concentration in the blood of dogs with LPE remains unclear. Lowe *et al.* showed that serum zinc concentration is unchanged between Irish setters with gluten-sensitive enteropathy and healthy ones [19]. In contrast, Grutzner *et al.* found that serum zinc concentration tends to be lower in Chinese Shar-Peis with marked hypocobalaminemia, which commonly presented with chronic gastrointestinal symptoms, than in those with normocobalaminemia [9]. Therefore, the purpose of this study was to investigate the serum zinc concentration in dogs with LPE and its influence on histological and clinical severities and prognosis.

Thirty-five dogs with LPE were recruited (Supplementary Table 1). These dogs presented with persistent gastrointestinal symptoms (at least 3 weeks) at the Veterinary Medical Center of Osaka Prefecture University, from April 2015 to July 2019. Endoscopic examination was performed for all dogs, and LPE was diagnosed based on histopathological evaluation using biopsy samples from the small intestine [6]. Puppies (less than 12 months old), dogs with severe concomitant diseases, and dogs with no stored serum samples were excluded. The included dogs comprised 7 intact males, 8 castrated males, 3 intact females, and 17 spayed females. The median age of dogs was 109 months (range, 13–161 months). The breeds included Miniature Dachshunds

(n=7), Shiba Inus (n=5), Chihuahuas (n=4), mixed breeds (n=4), French bulldogs (n=2), Miniature Schnauzers (n=2), Yorkshire Terriers (n=2), a Border Collie (n=1), a Bulldog (n=1), a Jack Russell Terrier (n=1), a Rottweiler (n=1), a Shetland Sheepdog (n=1), a Shih Tzu (n=1), a Siberian Husky (n=1), a Toy Poodle (n=1), and a Pembroke Welsh Corgi (n=1). Concomitant diseases presented by some of the dogs included pancreatitis (n=1), atypical Addison's disease (n=1), mitral regurgitation (n=1), cystitis (n=1), and Cushing's syndrome (n=1). When the blood samples were collected, the dogs were eating various diets, such as Gastrointestinal Low Fat (ROYAL CANIN, n=7), i/d low fat (Hill's-Colgate, n=4), w/d (Hill's-Colgate, n=3), i/d (Hill's-Colgate, n=2), Sensitive Control (ROYAL CANIN, n=2), Amino Protect Care (NOSAN, n=1), Chihuahua Adult (ROYAL CANIN, n=1), homemade (n=1), JP STYLE Clinista Digestive Organ Care (Nisshin Foods, n=1), Mini Adult (ROYAL CANIN, n=1), Pedigree (MARS, n=1), Poodle Adult (ROYAL CANIN, n=1), pH Control (ROYAL CANIN, n=1), Satiety Renovation (ROYAL CANIN, n=1), VITA-ONE (Nippon Pet Food, n=1), and unknown (n=7). Every commercial diet contained enough zinc, according to the association of American Feed Control Official (AAFCO) standards (zinc: >80 mg/kg) [4]. Sixteen dogs received prednisolone therapy (dose: 0.25–3 mg/kg/day) before the diagnosis. Based on a previous study [6], the histological severity of enteritis was graded as normal, mild, intermediate, or marked. Clinical severity was scored using canine IBD activity index (CIBDAI; score 0–15) and canine chronic enteropathy clinical activity index (CCECAI; score 0–20) [2, 14]. Informed consent was obtained from dog owners, for the use of clinical data and remaining blood samples for research and publication. This study did not reach the threshold for submission to a local ethical and welfare committee, since the collection and use of clinical data and serum samples were daily activities.

Plasma and serum samples were collected from each dog with LPE. Blood tests, including albumin and C-reactive protein (CRP), were performed for screening. The plasma albumin and CRP concentrations were measured using the bromocresol green and latex aggregation methods, respectively (FUJIFILM VET Systems Co., Ltd., Tokyo, Japan). The remaining serum samples were stored at −30°C, and serum zinc concentration was measured using atomic absorption spectrometry (FUJIFILM VET Systems Co., Ltd.) in January 2020. The reference ranges provided by the manufacturer of the examination device for blood albumin, CRP, and zinc concentrations were defined as 2.5–3.8 g/d*l*, <1.0 mg/d*l*, and 64–198 μg/d*l*, respectively.

For survival analyses, the status of each dog with LPE (alive, dead, or lost) until the end of the study (February 4, 2020) was obtained from medical records or fax interviews with the referring veterinarians. Overall survival (OS) was defined as the interval between the date of the diagnosis and the establishment of the cause of death at the end of the study.

The Kruskal–Wallis test and the Dunn's multiple comparison for a post-hoc analysis were performed to determine how serum zinc concentration was associated with appetite and histological severity in dogs with LPE. The Mann–Whitney U test was used to determine the difference in serum zinc concentration between dogs that had LPE (a) with and without hypoalbuminemia, and (b) with and without elevated CRP. The Spearman rank correlation was used to test associations of serum zinc concentration with CIBDAI and CCECAI scores in dogs with LPE. The Fisher's exact test was performed to determine the associations between serum zinc concentration and clinical characteristics in dogs with LPE. Survival curves were generated using the Kaplan–Meier method and compared using the log-rank test. Statistical analyses were performed with version 8.3.0 of Prism software, (Graph Pad Software Inc., San Diego, CA, USA). A P-value <0.05 was considered to be statistically significant.

The median serum zinc concentration in the dogs with LPE was 61 μg/d*l* (range, 19–100 μg/d*l*). Hypozincemia was observed in 18/35 (51%) cases. There was no significant association between serum zinc concentration and appetite in dogs with LPE (Supplementary Fig. 1). The median plasma albumin concentration in the dogs with LPE was 2.5 g/d*l* (range, 1.0–3.8 g/d*l*). Hypoalbuminemia was observed in 15/35 (43%) cases. The median serum zinc concentrations in dogs that had LPE with and without hypoalbuminemia were 51 μg/d*l* (range, 19–78 μg/d*l*) and 79 μg/d*l* (49–100 μg/d*l*), respectively (Fig. 1). There was a significant difference between the two groups (P=0.0001). The median serum zinc concentrations in dogs with LPE that received prednisolone therapy before the sample collection and those that did not were 59 μg/d*l* (range, 19–91 μg/d*l*) and 75 μg/d*l* (37–100 μg/d*l*), respectively; the difference was not significant.

The median plasma CRP concentration in the dogs with LPE was 0.12 mg/d*l* (range, 0.00–12.88 mg/d*l*). Elevated plasma CRP concentration was observed in 7/35 (20%) cases. The median serum zinc concentrations in dogs that had LPE, with and without elevated plasma CRP were 38 μg/d*l* (range, 19–61 μg/d*l*) and 76 μg/d*l* (35–100 μg/d*l*), respectively (Fig. 2). There was a significant difference between the two groups (P=0.0029). Hypozincemia was observed in all dogs with LPE that had elevated plasma CRP.

Based on histological severity, 4, 22, and 9 cases were classified as mild, intermediate, and marked LPE, respectively. The median serum zinc concentrations in dogs with mild, intermediate, and marked LPE were 70 μg/d*l* (range, 35–100 μg/d*l*), 76 μg/d*l* (range, 43–99 μg/d*l*), and 41 μg/d*l* (range, 19–73 μg/d*l*), respectively (Fig. 3). The serum zinc concentration was significantly decreased in dogs with marked LPE than in those with intermediate LPE (P=0.0068). There was no significant difference in the serum zinc concentration between the dogs with: (a) mild and intermediate LPE, and (b) mild and marked LPE. The serum zinc concentration in the dogs with LPE was inversely correlated with CIBDAI and CCECAI scores (CIBDAI: r_s= −0.5936, P=0.0002; CCECAI: r_s= −0.6430; P<0.0001; Fig. 4).

Thirty-four out of the 35 dogs with LPE were recruited for the survival analysis. These included 17 dogs with hypozincemia, and 17 without it. There was no significant difference in the age, sex, and neutered status between the two groups (Supplementary Table 2). During the study, 10/34 dogs died. The causes of death are shown in Supplementary Table 1. None of the dogs with LPE were euthanized. Out of the 10 dogs that died, 8 had hypozincemia. The median OS in the dogs that had LPE with and without hypozincemia was 574 days (range, 13−1,803 days) and 1,063 days (range, 19−1,990 days), respectively (Fig. 5). There was a significant difference in the OS between the two groups (P=0.0452).

The prevalence of hypozincemia in human patients with IBD ranges from 15% to 40% [1, 21, 23, 27, 31]. Common causes of
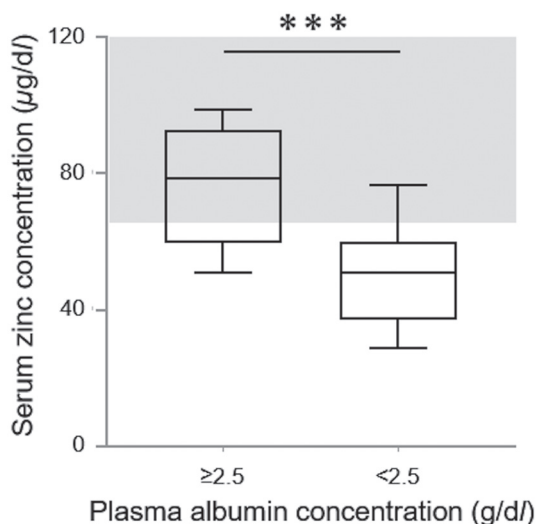
Fig. 1. Relationship between serum zinc and plasma albumin concentrations in dogs with lymphocytic-plasmacytic enteritis. The gray background indicates the reference range of the serum zinc concentration (64–198 μg/dl). The box plots show the 25th percentile (lower edge of the box), median (solid line in the box), 75th percentile (upper edge of the box), and 10–90th percentiles (whiskers). ***: $P<0.001$.
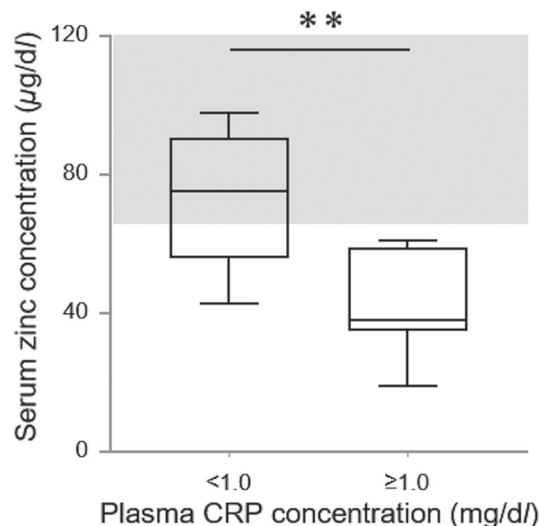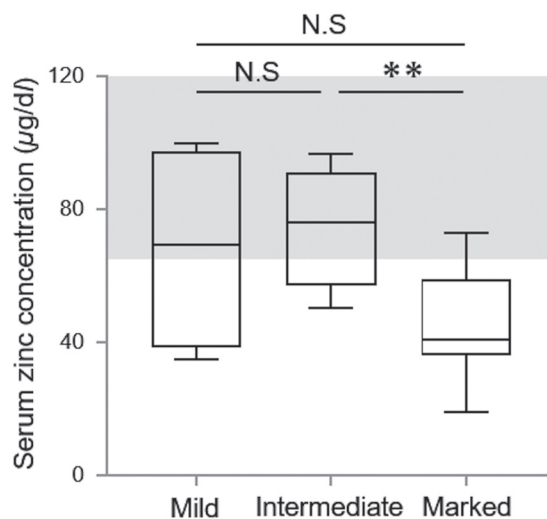

Fig. 2. Relationship between serum zinc and plasma C-reactive protein (CRP) concentrations in dogs with lymphocytic-plasmacytic enteritis. The gray background indicates the reference range of the serum zinc concentration (64–198 μg/dl). The box plots show the 25th percentile (lower edge of the box), median (solid line in the box), 75th percentile (upper edge of the box), and 10–90th percentiles (whiskers). **: $P<0.01$.
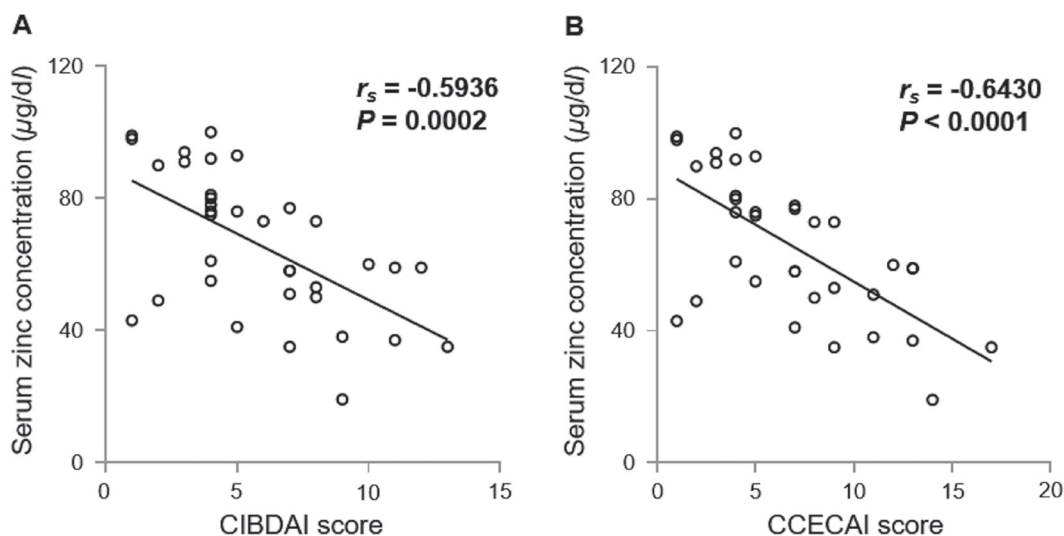
zinc deficiency include inadequate intake, decreased absorption, increased intestinal loss, and/or previous small bowel resection [1, 21. 23, 31]. Although there had been reports on the zinc concentration in the blood of dogs with LPE [19], the prevalence of hypozincemia was unclear. This study demonstrated that hypozincemia was observed in 51% of dogs with LPE. The cause, however, is still unclear. None of the dogs underwent small bowel resection. The dogs whose dietary zinc content could be examined had adequate intake, according the AACFO standards [4]. There was no significant association between the serum zinc concentration and appetite in the dogs with LPE. In contrast, the serum zinc concentration was significantly decreased in the dogs with LPE that had hypoalbuminemia than in those that did not. In addition, the serum zinc concentration in the dogs with LPE was inversely correlated with histological severity. These findings suggest that decreased absorption and/or increased intestinal loss may be a cause of zinc deficiency in dogs with LPE.

In dogs with LPE, the serum zinc concentration was significantly decreased in cases with elevated plasma CRP. Similarly, decreased serum zinc concentration in human patients with IBD has been associated with elevated serum CRP [27]. Preclinical models and human translational studies have demonstrated that zinc deficiency may exacerbate inflammation through disrupted epithelial barrier function, impaired mucosal immunity, and increased pro-inflammatory cytokines, such as tumor necrosis factor-alpha, interleukin-6, and interleukin-23 [10, 11, 13, 17, 20, 25, 26, 29, 30, 32]. Although the relationship between zinc deficiency and inflammation has not been reported in dogs with

Fig. 3. Association between serum zinc concentration and histological severity in dogs with lymphocytic-plasmacytic enteritis. Histological severity was graded as mild, moderate, or marked. The gray background indicates the reference range of the serum zinc concentration (64–198 μg/dl). The box plots show the 25th percentile (lower edge of the box), median (solid line in the box), 75th percentile (upper edge of the box), and 10–90th percentiles (whiskers). N.S., not significant. **: $P<0.01$.

LPE, our findings suggest that zinc deficiency may contribute to exacerbation of inflammation in the gastrointestinal tract.

A previous study has reported that zinc deficiency in human patients with IBD is associated with poor clinical outcomes, such as an increased risk of subsequent hospitalizations, surgeries, and disease-related complications [27]. In contrast, another previous study has shown that serum zinc concentration has no correlation with disease activity in human patients with IBD [22]. In dogs with LPE, OS was significantly shorter in cases with hypozincemia than in those without. It was unclear whether this was due to zinc deficiency or total
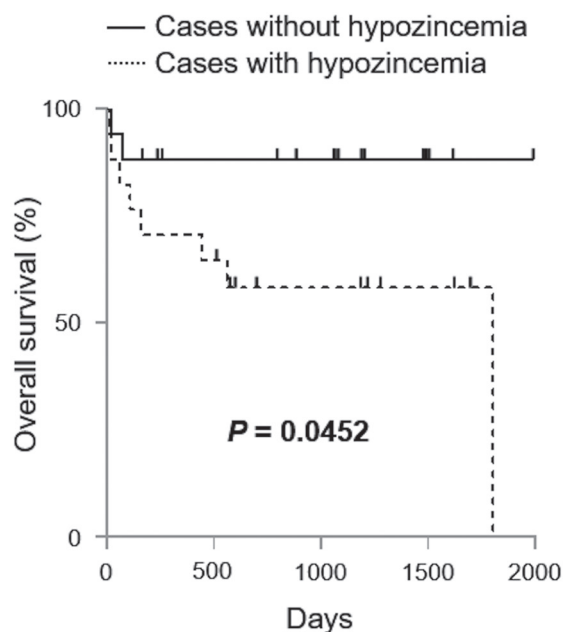
Fig. 4. Association between serum zinc concentration and clinical severity in dogs with lymphocytic-plasmacytic enteritis. The canine inflammatory bowel disease activity index (CIBDAI; score 0–15), and the canine chronic enteropathy clinical activity index (CCECAI; score 0–20) were used to determine the clinical severity.

severity of the disease. Moreover, the serum zinc concentration was inversely correlated with the histological and clinical severities. These findings suggest that serum zinc concentration in dogs with LPE is a useful biomarker for disease severity and prognosis. Although CIBDAI is very useful as a non-invasive method for assessing disease severity in dogs with LPE [14], an interview with the owner is required. The scores may also vary between individuals since activity, appetite, and stool consistency are subjectively evaluated. In contrast, the serum zinc concentration is objectively evaluated with the analyzer. The severity of LPE in dogs may be more accurately determined by assessing the serum zinc concentration in combination with CIBDAI.

A prospective study, examining 170,776 healthy women who were followed-up over a 26-year period, reported that dietary zinc supplementation is inversely associated with the risk of Crohn's disease [3]. Moreover, zinc supplementation has also resolved small intestinal permeability in human patients with Crohn's disease in remission [28]. Endoscopic scores of human patients with ulcerative colitis after zinc supplementation improved [12]. Zinc supplementation may be effective for prevention and treatment of human IBD. However, the effect of zinc supplementation on dogs with LPE is unknown. Therefore, prospective studies are required to investigate the benefits of zinc supplementation in dogs with LPE.

This study had limitations. First, data for some dogs were not available because of the retrospective design of the study. Second, diets varied among the dogs, and this might have influenced the serum zinc concentration. However, the diets with zinc content

Fig. 5. Kaplan–Meier curves of overall survivals in dogs with lymphocytic-plasmacytic enteritis in relation to hypozincemia. Among the included dogs, 17 had hypozincemia while 17 did not. The black symbols indicate censored cases.

that could be examined met the AAFCO standards [4]. Third, treatments after the diagnosis of LPE were not unified among the dogs, and this may have affected OS. Further prospective studies with unified diet and treatments after diagnosis are warranted. Fourth, changes in the serum zinc concentrations along with treatments were not evaluated because there were no dogs with serum samples stored during the followed-up period.

In conclusion, the present study demonstrated the presence of hypozincemia in dogs with LPE. The serum zinc concentration was inversely correlated with histological and clinical severities in dogs with LPE. Additionally, dogs with LPE that had hypozincemia had poor prognosis. These findings suggest that the serum zinc concentration is a useful biomarker for LPE severity and prognosis in dogs.

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