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Assessment of intracellular zinc levels in infants with food protein-induced allergic proctocolitis

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Abstract
Background: Food protein-induced allergic proctocolitis (FPIAP) is characterized by bloody stools in well-appearing infants. Zinc is a micronutrient that plays a crucial role in immune modulation and is essential for cellular function during immune response. Although there are studies on the assessment of intracellular zinc levels in allergic diseases, no data is available on erythrocyte zinc levels of patients with FPIAP.
Objective: This study aimed to assess the erythrocyte zinc levels of children with allergic proctocolitis and compare zinc levels with clinical and demographic characteristics.
Methods: This was a case-control study that prospectively compared 50 patients with FPIAP and 50 healthy children without malnutrition. The erythrocyte zinc levels of children were determined using atomic absorption spectrophotometry.
Results: Fifty patients with FPIAP, including 28 (51%) girls, with median age of 7.1 ± 2.9 (3-14) months and 50 healthy children, including 26 (53.1%) girls, with median age of 7.7 ± 2.8 (3-13) months were included in the study. Seventy percent (n = 35) of the patients with FPIAP started to have symptoms while they were exclusively breastfeeding. Offending allergen foods were cow's milk (78%), egg (40%), sesame (10%), hazelnut (8%), almond (6%), beef (6%), and peanuts (6%, n = 3). Intracellular (erythrocyte) zinc levels in patients with FPIAP were lower than in the healthy control group (495.5 ± 134 µg/dL, 567.3 ± 154.4 µg/dL, respectively, P = 0.01). Patients with FPIAP aged younger than 6 months had lower intracellular zinc levels compared with those aged above 6 months (457 ± 137 µg/dL; 548 ± 112 µg/dL, respectively, P = 0.01). There was no relationship between zinc levels and time of symptom onset, presence of concomitant disease, being allergic to multiple foods, and family history of atopy (P > 0.05).
Conclusions: FPIAP is a food allergy with limited information on its pathogenesis. Considering the beneficial effects on gastrointestinal system epithelia, zinc may be involved in the pathogenesis of FPIAP. Future comprehensive prospective research on this subject is of importance.

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Introduction

Food protein-induced allergic proctocolitis (FPIAP) is a non-immunoglobulin (Ig)-E mediated food allergy.[1] FPIAP is characterized by inflammatory changes in the rectum and distal sigmoid colon, resulting from an immune response triggered by the ingestion of allergen food proteins.[2-4] FPIAP is a benign disease characterized by bloody stool, which usually begins in the first months of life in well-appearing infants, and it is a major cause of colitis in babies aged younger than 1 year.[1,5,6] FPIAP accounts for 0.16–64% of isolated rectal bleeding in infants.[7,8]

FPIAP is characterized by the intermittent passage of bloody, mucousy, frothy, and watery stools that usually starts before the sixth month of age.[9-11] Its diagnosis is based on patient history and relief of symptoms after elimination of suspected allergen food from the diet and lacks confirmatory diagnostic tests except for oral food challenge (OFC).[12] It can be associated with high serum IgE levels and eosinophilia in peripheral blood.[9-13] A specific laboratory test for diagnosis is not available, and skin prick tests and food-specific IgE tests at the time of presentation are found negative in most patients.

The pathophysiology of the disease has not been fully elucidated. It has been reported that the eosinophilic infiltration in immature intestines of infants plays a role in the pathogenesis of FPIAP, and increased intestinal permeability caused by eosinophilic degranulation and disruption of the intestinal tight junction barrier directly causes FPIAP symptoms when a food allergen is encountered.[1,14]

Zinc is a trace element that is known to affect epithelial barrier function and plays a regulatory role in the immune system. It is important to maintain the activities of the signal molecules of many enzymes.[15] Zinc is also an antioxidant molecule.[16] It takes part in immune system mechanisms such as cell differentiation and proliferation.[17] It has been reported that processes such as T cell activation and T helper cell differentiation are affected by zinc homeostasis. Maywald et al. reported the increased production of proinflammatory cytokines and the shift in T cell balance toward T helper 2 cells in mild zinc deficiency.[17]

The majority of free zinc is located within cells, and the serum zinc pool represents approximately 1% of the total body content.[18] Intracellular zinc measurement is accepted as a good marker for predicting body zinc levels and a better test to detect mild zinc deficiency.[19] Low erythrocyte zinc levels have been reported in many allergic diseases such as asthma and atopic dermatitis.[20,21]

The literature lacks studies investigating the intracellular zinc levels of children with allergic proctocolitis. This study aimed to assess erythrocyte zinc levels of children with allergic proctocolitis and compare them with healthy children.

Method

Study population

This was a prospective case-control study that compared 50 children with FPIAP and 50 healthy children without malnutrition who were paired for age and sex. According to the European Academy of Allergy and Clinical Immunology (EAACI) guidelines, patients were diagnosed as having FPIAP if, they presented with a bloody stool after the ingestion of a possible trigger food, responded positively to the elimination of the suspected food, and their symptoms reappeared when an OFC was performed.[1]

Patients with missing data; patients without a definitive FPIAP diagnosis confirmed with OFC; patients with diseases causing bloody stool, including anal fissure, perianal dermatitis or excoriation, invagination, coagulopathies, necrotizing enterocolitis, inflammatory bowel diseases, vitamin K deficiency, and immune deficiency; and patients with underlying chronic gastrointestinal diseases were excluded from the study.The study was approved by the local ethics committee (2020.160.06.22), and informed consent was obtained from all parents or guardians.

Skin prick testing and evaluation of food-specific IgE

Skin prick test and serum food-specific IgE evaluations were performed on patients to detect food allergen sensitivity. The total serum IgE and food-specific IgE (sIgE) levels in the serum were measured using a IMMULITE 2000 XPi (Siemens Healthineers, Erlangen, Germany). Specific IgE titers were quantified in protein units designated as kilounits of antibody per liter (kU/L); IgE ≥ 0.35 was considered positive.[22] Skin prick tests were performed on all patients with suspected foods including cow's milk, soy, egg white, egg yolk, wheat, walnut, hazelnut, peanut, sesame, and almond using commercial extracts (ALK-Abello A/S, Horsholm, Denmark standard prick test solutions). Histamine (10 mg/mL) was used as positive control, and NaCl (0.9%) was used as negative control. A wheal size ≥ 3 mm, larger than the negative control, was accepted as positive.

Oral food challenge (OFC)

All patients underwent OFCs to confirm the diagnosis of FPIAP after 2 weeks in non-severe and 4 weeks in severe cases following the resolution of symptoms after the elimination of trigger foods. OFCs were performed on breastfed infants to confirm the diagnosis following symptom resolution after removing the trigger food from the mother's diet.[23,24] OFCs were implemented on these patients through breastfeeding, which indicated the presence of FPIAP.

The weights and heights of the children were measured using the same device, and their weight-to-height ratios were calculated. Malnutrition was assessed using standard charts for Turkish children. A weight-to-height ratio of 80–90% was considered as mild malnutrition, and a weight-to-height ratio above 90% was considered normal.

Technique for measuring zinc (in erythrocytes)

Fasting blood samples were taken in vacutainer blood sampling tubes containing K3EDTA and no additive, between 08:00 and 10:00 AM. The blood samples for the measurement of erythrocyte zinc was taken the following morning after the OFC. Zinc measurements were made using an

atomic absorption spectrophotometer (Perkin Elmer AAS Flame PinAAcle 900F, Perkin Elmer Inc., MA, USA) utilizing the absorption spectrophotometry method.[25] Erythrocyte zinc levels are expressed as microgram/dL.

Statistical method

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, Chicago, Illinois, USA), Version 23.0 package software. Descriptive statistics were expressed as mean, standard deviation, median, minimum and maximum values, and percentages. A chi-squared test was used in the analysis of the categorical data. A normal distribution test was performed on all data. For the comparison of quantitative data, Student's t-test was used for those with normal distribution, and Mann–Whitney U test was used for nonparametric data. $P < 0.05$ was considered statistically significant.

Results

Fifty patients with FPIAP with a mean age of 7.1 ± 2.9 (range: 3–14) months were included in the study. Twenty-eight (56%) patients were girls. The mean age of the healthy control group was 7.7 ± 2.8 (range: 3–13) months, and 53.1% (n = 26) were girls. There was no difference in age (P = 0.14) and gender (P = 0.84) between the patients with allergic proctocolitis and healthy controls. The mean age of the patients with allergic proctocolitis at symptom onset was 3.4 ± 1.6 (range: 0.5–6) months. Twenty-three (46%) patients had concomitant diseases, which consisted of atopic dermatitis (26%, n = 13), gastroesophageal reflux (18%, n = 9), and wheezing (2% n = 1). Of the patients diagnosed as having allergic proctocolitis, 68% (n = 34) were delivered by cesarean, and 32% (n = 16) by spontaneous vaginal delivery. A family history of allergic diseases was present in 28% (n = 14) of the patients. The mean period of exclusive breastfeeding was 4.2 ± 1.5 (range: 0.5–8) months, and 70% (n = 35) started to have symptoms while they were exclusively breastfeeding (Table 1).

Symptoms of patients

The main symptom of all patients was rectal bleeding with different amounts of mucous. In addition, 48% (n = 24) of the patients had noisy bowel movements, 52% (n = 26) had green stools, 42% (n = 21) had difficulty in defecation, 24% (n = 12) had large amounts of watery stool, 18% (n = 8) had constipation, and 14% (n = 7) had diaper dermatitis. Repetitive vomiting was reported in 12% (n = 6) of the patients. Twenty-five (50%) out of the 50 patients had accompanying symptoms other than gastrointestinal symptoms. Those were atopic dermatitis (26%, n = 13), nocturnal cough (20%, n = 10), and failure to thrive (4%, n = 2).

Offending foods

Offending allergen foods were cow's milk (78%, n = 39), egg (40%, n = 20), sesame (10%, n = 5), hazelnut (8%, n = 4), almond (6%, n = 3), beef (6%, n = 3), and peanut (6%, n = 3). Some of the patients with cow's milk allergy were also allergic to egg (28.2%, n = 11), nuts (15.3%, n = 6), or beef (10%, n = 4). Food sensitization was diagnosed in 28% of the patients, including sensitization to egg white (n = 10), cow's milk (n = 8), egg yolk (n = 2), sesame (n = 3), hazelnut (n = 4), almond (n = 4), peanut (n = 3), and walnut (n = 1). Twenty percent of the patients had FPIAP symptoms triggered by more than one food.

Complete blood count and biochemical parameters were normal in all patients (Table 2). Twenty-four percent (n = 12) of the patients had neutropenia (<1500/mm^3), and 16% had eosinophilia (>500/mm^3).

Intracellular (erythrocyte) zinc levels were 495.5 ± 134 (290–848) μg/dL for patients with allergic proctocolitis and 567.3 ± 154.4 (202–820) μg/dL for healthy controls; Zn levels were lower in patients with FPIAP (P = 0.01) (Figure 1).

Table 1 Clinical and demographic features of patients with allergic proctocolitis.

Mean age	7.1 ± 2.9 (3–14) months
Gender	56% (n = 28) females, 44% (n = 22) males
Mode of delivery	68% (n = 34) cesarean delivery, 32% (n = 16) spontaneous vaginal delivery
Exclusive breastfeeding period	4.2 ± 1.5 (0.5–8) months
Concomitant diseases	46% (n = 23) atopic dermatitis: 26% (n = 13) gastroesophageal reflux: 18% (n = 9) wheezing: 2% (n = 1)
Time of symptom onset	3.4 ± 1.6 (range: 0.5–6) months 70% (n = 35) while exclusively breastfeeding
Patient with more than one FPIAP trigger food	20% (n = 10)
Presence of food sensitization	28% (n = 14)
Presence of family history of atopy	28% (n = 14)

FPIAP: Food protein-induced allergic proctocolitis

Table 2 Laboratory results of patients with allergic proctocolitis.

Total IgE (median) (IU/L)	49.5 (1.15-875)
WBC (/mm^3)	8849 ± 2728 (5090-14970)
Neutrophil (/mm^3)	2214 ± 1018 (990-6140)
Lymphocyte (/mm^3)	5456 ± 1771 (970-9680)
Eosinophil (/mm^3)	329.2 ± 249 (260-1070)
Eosinophil (%)	3.8 ± 2 (0.3-9.6)
Iron (ug/dL)	56.1 ± 27 (19-124)
Cow's milk specific-IgE (median) (kU/L)	0.28 (0-35)
Egg white specific-IgE (median) (kU/L)	0.2 (0.1-6.25)
Egg yolk specific-IgE (median) (kU/L)	0.1 (0.1-6.21)

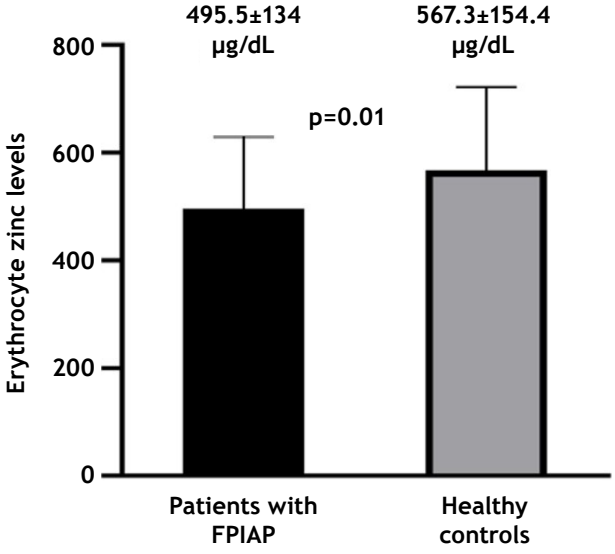

Figure 1 Erythrocyte zinc levels for the patients with allergic proctocolitis and healthy control group.

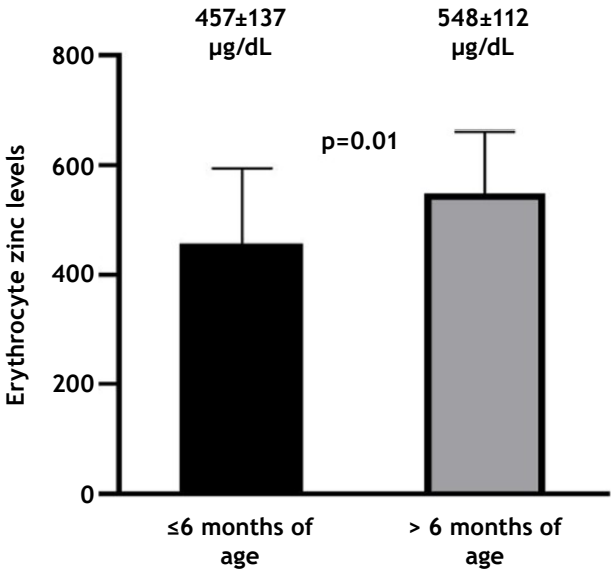

Figure 2 Erythrocyte zinc levels for patients with allergic proctocolitis according to age.

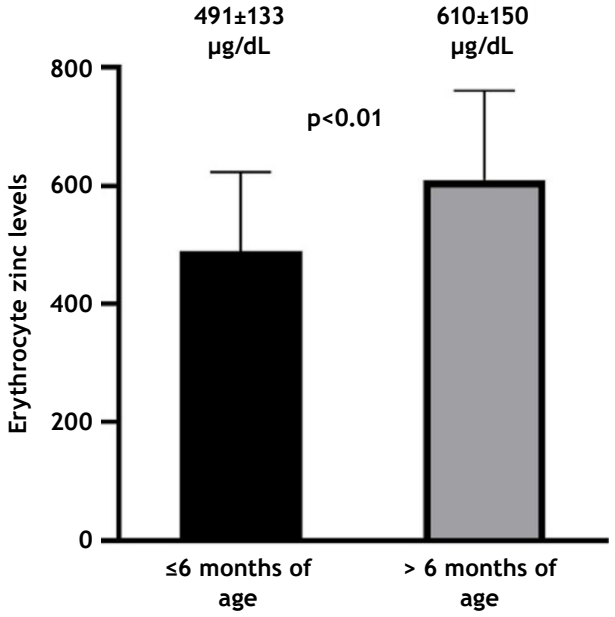

Figure 3 Erythrocyte zinc levels for healthy control group according to age.

Patients with allergic proctocolitis aged younger than 6 months had lower erythrocyte zinc levels compared with those aged above 6 months (457 ± 137 [290-848] µg/dL and 548 ± 112 [338-786] µg/dL, respectively, P = 0.01) (Figure 2). Children in healthy control groups aged younger than 6 months had lower erythrocyte zinc levels compared with those aged above 6 months (491 ± 133 [300-804] µg/dL and 610 ± 150 [201-820] µg/dL, respectively, P < 0.01) (Figure 3).

There was no difference in zinc levels between patients with and without concomitant diseases (n = 23 and n = 27, 523 ± 122 µg/dL and 471.8 ± 141 µg/dL, respectively, P = 0.17). There was no difference in zinc levels between patients with and without atopic dermatitis (P = 0.13) or gastroesophageal reflux (P = 0.68) (Table 3). Zinc levels did not differ according to the presence of food sensitization, multiple food allergies, or family history of atopy (Table 3).

There was no difference in zinc levels between the different times of symptom onsets, including the periods of exclusive breastfeeding (n = 35), feeding with breast milk and formula (n = 12), complementary feeding (n = 3) (489 ± 129 µg/dL, 519.8 ± 163 µg/dL, and 469.7 ± 47 µg/dL, respectively, P = 0.87). The zinc levels were similar in patients who were exclusively breastfeeding and patients who fed with formula or complementary food with or without breast milk (449.5 ± 110 µg/dL and 511.6 ± 139 µg/dL, respectively, P = 0.15).

Table 3 Comparison of erythrocyte zinc levels according to the presence of concomitant diseases, food sensitization, number of trigger food, and family history of atopy.

	Erythrocyte zinc level (μg/dL)		
	Yes	No	P
Concomitant disease	523 ± 122 (333–848) (n = 23)	471.8 ± 141 (290–806) (n = 27)	0.17
Atopic dermatitis	533.8 ± 124 (333–848) (n = 13)	473.9 ± 135 (290–806) (n = 37)	0.13
Gastroesophageal reflux	512 ± 130 (338–770) (n = 9)	491 ± 136 (290–848) (n = 41)	0.68
Food sensitization	518.8 ± 134 (290–848) (n = 14)	472 ± 132 (307–806) (n = 36)	0.22
> One trigger food	553 ± 147 (333–848) (n = 10)	481 ± 128 (290–806) (n = 40)	0.13
Family history of atopy	530 ± 125 (389–806) (n = 14)	478 ± 144 (290–848) (n = 36)	0.24

Discussion

FPIAP is a food allergy with limited information on its pathogenesis. Zinc is a micronutrient that plays a crucial role in immune modulation and is essential for cellular functions during immune response. Studies investigating zinc levels in allergic diseases have been increasing. Despite the presence of studies investigating erythrocyte zinc levels in allergic diseases such as atopic dermatitis, the data on zinc levels in FPIAP are lacking. To the best of our knowledge, this is the first study to investigate zinc levels in erythrocytes of patients with FPIAP.

Kamer et al. reported that the children with food allergies had low zinc levels and weak antioxidant barriers.[26] It was also reported that the erythrocyte zinc levels were low in allergic diseases associated with epithelial barrier injuries such as asthma and atopic dermatitis.[20-21] Maares et al. reported that zinc supplementation had potential benefits such as anti-inflammatory and immunomodulatory effects in the prevention of allergies and autoimmunity.[27] We assessed the zinc levels in allergic proctocolitis, in which intestinal permeability increases for allergen food, and found them to be lower compared with healthy children. Zinc is an important nutrient in the early life diet. In patients with allergic proctocolitis, patients aged younger than 6 months had lower zinc levels compared with patients aged above 6 months (457 ± 137 and 548 ± 112 μg/dL, respectively).

Zinc is a trace element that is known to affect epithelial barrier function. Studies in animal models reported the impact of pharmacologic levels of Zn supplementation on tight junction proteins, and Zn supplementation reduced intestinal permeability in diarrhea.[28,29] Measurement of zinc levels in serum is the most commonly used zinc measurement technique.[30] In a study of children with allergic colitis, low levels of zinc, selenium, and copper were found.[31] However, zinc levels in serum are influenced by a variety of factors such as infection, stress, and low albumin levels.[32] Intracellular zinc level measurements have been reported to be a good method to determine zinc deficiency.[19] However, the association between allergic proctocolitis and erythrocyte zinc levels has not yet been known.

More than 60% of the patients with allergic proctocolitis are breastfeeding children.[33] Symptoms of the disease usually start at 2–8 weeks of life.[2] In this study, similar to the literature, symptoms occurred at the exclusive breastfeeding period in 70% of patients, and the mean time of symptom onset was 3.4 months. The zinc levels were similar in patients who were exclusively breastfeeding and/or receiving formula or complementary food with or without breastmilk.

Cow's milk is responsible for most FPIAP symptoms, but other foods such as soy, egg, wheat, and fish have also been associated with the disease.[3] In the study by Erdem et al., offending foods were cow's milk for 78% of patients, milk and egg for 13%, and egg for 5%.[34] In this study, the most common offending foods were cow's milk (78%) and egg (40%).

It was reported that most patients with FPIAP did not have IgE-mediated food sensitization.[1,3,35] Neverthless, Çetinkaya et al. showed that there is evidence of IgE sensitization to offending foods in 19.4% of the patients.[36] Food sensitization rate was even higher in our study, which involved 28% of the patients. The percentage of having a family history of atopy was 64% as per the study by Lucarelli et al.; however, it was as low as 28% in our study.[37] No difference was found between the zinc levels according to food sensitization and family history of atopy.

Different studies have reported rates of FPIAP triggered by multiple foods as 3.3 and 5%.[5,38] In this study, the rate of symptoms with multiple foods was found to be as high as 28%. Nowak-Wegrzyn et al. reported that FPIAP was accompanied by increased gas (30%), intermittent vomiting (27%), pain on defecation (22%), and abdominal pain (20%).[6] In our study, it was accompanied mostly by noisy bowel movements (48%), as well as straining or difficulty in defecation (24%), constipation (18%), diaper dermatitis (14%), and repetitive vomiting. All patients with vomiting also had concomitant gastroesophageal reflux disease. There was no relation between zinc levels and presence of accompanying symptoms or concomitant diseases.

Nowak-Wegrzyn et al. also reported peripheral eosinophilia in FPIAP.[6] In the present study, 16% of the patients had eosinophilia, and there was no relation between zinc levels and presence of eosinophilia.

Zinc deficiency is known to cause epithelial barrier leakage in the gastrointestinal tract.[39] In their in vitro gastrointestinal model, Wang et al. reported that zinc supplementation could be used to prevent tight junction leakiness in various diseases through its potential tight junction modifier and epithelium strengthening effects.[39] Patients with newly diagnosed inflammatory bowel disease, which is a gastrointestinal disease associated with epithelial barrier

injury, were reported to have low zinc levels.[40] Zinc deficiency is a significant public health issue in many developing countries. Also, the World Health Organization and United Nations Children's Fund (UNICEF) reported that zinc decreased the severity and duration of acute diarrhea in children, and recommend zinc supplementation for children with diarrhea.[41]

In our study, patients with FPIAP were found to have lower zinc levels; considering the improving effect of zinc on the intestinal barrier. This suggested that low intracellular zinc levels might have a role in the pathogenesis of FPIAP.

The strengths of this study are that it is a prospective study that compared patients with a healthy control group and that erythrocyte zinc measurements were used, which is a favorable parameter to assess zinc levels.

A limitation of our study was that the diet of the mother and daily zinc consumption was neglected. However, the weights and heights of the patients were in the normal range for their ages and none had malnutrition, which could cause zinc deficiency.

In conclusion, we detected lower zinc levels in the erythrocytes of children with allergic proctocolitis than in the healthy control group. Considering the beneficial effects of zinc on gastrointestinal system epithelia, zinc is thought to be involved in the pathogenesis of FPIAP. Future comprehensive prospective studies are important to understand the role of zinc levels in the occurrence or course of allergic proctocolitis.

Statement of Ethics

The study was approved by the Local Ethics Committee (Tekirdağ Namık Kemal University Ethics Committee, 2020.160.06.22).

Conflict of Interest

No conflict of interest was declared by the authors.

Financial Disclosure

The authors declared that no financial support was received for this study.

Author Contributions

NCG designed the research, followed up with patients, performed data collection, wrote the paper, and analyzed the results. AC designed the methodology and performed data collection. AN followed up with patients and performed data collection. All three authors contributed to the editing of the manuscript.

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