The long-term clinical course of protein-losing enteropathy combined with iron deficiency anemia in Korean toddlers: Possible association with cow’s milk protein

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**Key Words**
child; cow’s milk protein allergy; edema; iron deficiency anemia; protein-losing enteropathy

**Background:** Protein-losing enteropathy (PLE), a rare condition with excessive gastrointestinal protein loss, presents with hypoalbuminemia, edema, or ascites. Several cases of PLE combined with severe iron deficiency anemia (IDA) have been reported in infants and toddlers that were considered to result from excessive cow’s milk consumption, although the mechanism has not been clearly established.

**Methods:** We retrospectively reviewed the clinical, laboratory, endoscopic, and radiologic characteristics of patients diagnosed and treated for PLE with IDA between 2015 and 2021. Long-term outcomes were analyzed according to dietary intervention during the follow-up period.

**Results:** A total of 10 patients aged 7.0–26.7 months were enrolled in the study and the median follow-up duration of them was 9.4 months (range, 1.3–18.0). Six of them were fed powdered formula, while two were fed whole cow’s milk, and their median daily intake was 700 mL (range, 300–900). The times to normalization of hemoglobin, albumin, and eosinophil count were shorter in patients with dietary elimination of cow’s milk protein immediately after diagnosis compared to those with reduced intake or no dietary change.

**Conclusion:** Early complete elimination of cow’s milk protein should be considered, especially if the laboratory parameters are not normalized with adequate iron supplementation even though the clinical symptoms show improvement. We would like to draw attention to the...
possibility of the cow’s milk protein in the pathogenesis of the condition through the non-IgE-mediated immune reactions.

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1. Introduction

Protein-losing enteropathy (PLE) is a rare condition in which excess proteins are lost through the gastrointestinal (GI) tract, leading to hypoalbuminemia, edema, ascites, as well as pleural or pericardial effusion. The causes of PLE are categorized into erosive or non-erosive mucosal injury of the GI tract and increased lymphatic pressure caused by diverse systemic diseases.

Iron deficiency (ID) is the most common nutritional deficit in children worldwide. Even in healthy infants born with sufficient iron stores from the mother, rapid growth during infancy deplete their iron reserves by 4–6 months, increasing the risk of ID in late infancy. Thus, iron fortification during infancy is essential to avoid iron deficiency anemia (IDA) in toddlers.

Several cases of healthy infants or toddlers identified with PLE combined with IDA have been reported. Although most cases have been suggested to be related to excessive consumption of cow’s milk, the exact mechanism of the association between PLE and IDA has not been elucidated. Moreover, the treatment protocol or preventive strategies have not been determined other than supportive management. Therefore, we aimed to determine the cause of PLE combined with IDA and to suggest appropriate assessment and management strategies by describing the long-term clinical course of previously healthy Korean infants and toddlers diagnosed with PLE combined with IDA.

2. Materials and methods

We retrospectively reviewed the electronic medical records of patients diagnosed with and treated for PLE with IDA between May 2015 and Dec 2021 in two National University Hospitals of South Korea. PLE was diagnosed when the stool α1-antitrypsin (A1AT) level was >54 mg/dL in a screening test together with the presence of hypoalbuminemia in patients with clinical symptoms. In this study, we only included patients diagnosed with PLE and IDA after excluding the patients who were diagnosed with underlying diseases such as heart diseases, intestinal lymphangiectasia, Crohn’s disease, intestinal infection, or severe malnutrition that presents as PLE after an extensive diagnostic work-up.

To identify the cause of hypoalbuminemia during the diagnosis of PLE, urinalysis, stool culture, stool parasite, fecal calprotectin, stool occult blood, liver function tests, thyroid function test, or esophagogastroduodenoscopy (EGD), colonoscopy (CFS), abdominal ultrasonography, abdominal computed tomography (CT), or echocardiography were performed when indicated. In patients with a history of febrile illness within 4 weeks, serum and urine cytomegalovirus (CMV) polymerase chain reaction (PCR) were performed in addition to the respiratory viral panel to rule out Menetrier’s disease, a rare cause of PLE in toddlers.

The IDA was defined as a hemoglobin (Hb) level of <11.0 g/dL along with concurrent ID. ID was diagnosed when the serum ferritin level was <12 ng/mL, mean corpuscular volume (MCV) was <70 fL, and transferrin saturation was <10%. In patients with peripheral eosinophilia at presentation, serum total IgE and food-specific IgEs were measured to identify any food hypersensitivity.

Clinical and demographic information was documented, including the initial presenting symptoms, past medical history such as recent febrile illness, and current medication. Information regarding the current types of feeding (e.g., breastfeeding, formula feeding, whole cow’s milk feeding), the daily amount of formula or whole cow’s milk intake, and dietary habits or adequacy of food intake were also obtained from the parents/guardians. The treatment regimens, including dietary intervention, if present, were collected. The results of serial laboratory testing performed from initial presentation through the follow-up period were also collected. The time to recovery of the Hb level to ≥11.0 g/dL, albumin level ≥3.4 g/dL, and eosinophil count ≤650 cells/μL was calculated, and defined as the time to normalization of the laboratory parameters.

According to the time to start complete elimination of cow’s milk-based foods, the patients were divided into groups for analysis. When cow’s milk-based formula or milk was completely restricted immediately after the diagnosis of PLE and IDA, the patients were classified as the early dietary intervention group. When cow’s milk-based foods were completely eliminated several weeks or months after diagnosis, the patients were classified as the late intervention group.

All analyses were performed using PASW software (version 23.0; SPSS Inc., Chicago, IL, USA) with a statistical significance level of \( p < 0.05 \). Non-normally distributed continuous variables were expressed as medians and ranges and were compared using the Mann–Whitney test. The study was conducted in accordance with the Declaration of Helsinki, and approved by the appropriate Institutional Review Board (IRB No. 05-2021-139). The requirement for informed consent was waived due to the retrospective nature of the study.

3. Results

3.1. Demographic characteristics of the patients

A total of 10 patients (eight boys, two girls) were identified during the study period (Table 1). The median age at
Seven patients underwent the serum total IgE and food-sensitive IgE test. Eight patients underwent the fecal occult blood test. Among the nine patients whose dietary information could be obtained, there were no exclusively breastfed infants (Table 2). Six of them were fed with formula milk, while two were fed with whole cow’s milk, and their median daily intake was 700 mL (range, 300–900). One patient did not consume either formula or whole cow’s milk. A 9-month-old patient was fed with goat’s milk-based formula since birth according to the parent’s preference. Two patients continued the formula without switching to whole cow’s milk even beyond 12 months of age. One of them, a 20-month-old patient, had been reported to have feeding problems, refusing to take solid foods and continuing bottle feeding with formula without switching to whole milk. However, the parents or caregivers of the other patients did not report any feeding difficulties or food intolerance in their children.

### 3.2. Laboratory, endoscopic, and radiologic findings of the patients

At the time of diagnosis, the median Hb and albumin levels of the patients were 6.0 g/dL (range, 4.3–9.3) and 2.2 g/dL (range, 1.8–2.6) respectively (Table 1). Except for three patients, all patients showed peripheral eosinophilia (>500 cells/µL) at diagnosis. The median eosinophil count was 1010 cells/µL (range, 120–3320). Among the patients with peripheral eosinophilia, only one patient showed high levels of total IgE and specific-IgE to cow’s milk. None of the patients had a positive serum or urine CMV PCR result and 75% had a positive stool occult blood test.

Among the eight patients who underwent abdominal ultrasonography or CT, small bowel wall thickening was found in five patients, while the others exhibited non-specific findings. EGD was performed in four patients and showed nodular lymphoid hyperplasia, mainly in the duodenal bulb in three patients with grossly normal gastric mucosa. On histologic examination, no abnormalities were found, such as significant eosinophil infiltration. CFS was performed in two patients and showed mild nodular lymphoid hyperplasia in the colonic mucosa; however, there were no evident mucosal lesions responsible for obscure GI bleeding such as ulcers or erosions. The clinical, laboratory, endoscopic, and radiologic characteristics of each patient at diagnosis are presented in Table S1.

### 3.3. Clinical course during follow-up

The median follow-up duration of the patients was 9.4 months (range, 1.3–18.0 months). Half of the patients received packed red blood cell (RBC) transfusions and albumin infusions, respectively. Three patients were not treated with RBC transfusion or albumin infusion. Oral iron supplements were administered to all patients immediately after the diagnosis of IDA.

The normalization of Hb levels took longer than that of albumin levels. The median time to normalization of Hb and albumin levels was 4.2 months (range, 0.7–8.3) and 2.1 months (range, 0.7–8.2), respectively. The eosinophil count took the longest time to normalize, with a median of 7.7 months (range, 1.3–14.0).

Dietary modulation was tried in eight patients. Three patients were changed to an amino acid-based formula (Neocate®). Two patients older than 12 months were advised to discontinue feeding with their current infant formula and to increase their regular diet. In two patients who were fed whole cow’s milk, complete elimination of the milk intake and other dairy foods was recommended. In the case of the 27-month-old boy who did not take either...
Table 2  Clinical course of the patients post-intervention during the follow-up period.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (months)/ Sex</th>
<th>Current feeding type</th>
<th>CM intake (mL/day)</th>
<th>RBC</th>
<th>Albumin</th>
<th>Diet modification/Time to start</th>
<th>Time to normalization (months)</th>
<th>Albumin (≥3.4 g/dL)</th>
<th>Eosinophil count (≤500/μL)</th>
<th>Total follow-up duration (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7/M</td>
<td>FF, WF</td>
<td>900</td>
<td>N</td>
<td>Y</td>
<td>Change to AAF/After 0.7 months</td>
<td>1.2</td>
<td>1.2</td>
<td>7.7</td>
<td>7.7</td>
</tr>
<tr>
<td>2</td>
<td>9/F</td>
<td>FF, WF</td>
<td>800</td>
<td>Y</td>
<td>Y</td>
<td>Change to AAF/At diagnosis</td>
<td>0.7</td>
<td>0.7</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>3</td>
<td>12/M</td>
<td>BMF, FF, WF</td>
<td>600</td>
<td>N</td>
<td>N</td>
<td>ND</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>4</td>
<td>12/M</td>
<td>FF, WF</td>
<td>600</td>
<td>N</td>
<td>Y</td>
<td>Quit formula/After 5.1 months</td>
<td>5.9</td>
<td>8.2</td>
<td>13.0</td>
<td>13.0</td>
</tr>
<tr>
<td>5</td>
<td>13/F</td>
<td>FF, WF</td>
<td>300</td>
<td>Y</td>
<td>N</td>
<td>ND</td>
<td>5.8</td>
<td>7.7</td>
<td>9.3</td>
<td>18.0</td>
</tr>
<tr>
<td>6</td>
<td>14/M</td>
<td>WMF, WF</td>
<td>800</td>
<td>N</td>
<td>N</td>
<td>Quit WMF/At diagnosis</td>
<td>2.1</td>
<td>0.7</td>
<td>7.6</td>
<td>9.7</td>
</tr>
<tr>
<td>7</td>
<td>14/M</td>
<td>NR</td>
<td>NR</td>
<td>N</td>
<td>N</td>
<td>Change to AAF/After 3.0 months</td>
<td>8.3</td>
<td>3.3</td>
<td>14.0</td>
<td>14.0</td>
</tr>
<tr>
<td>8</td>
<td>20/M</td>
<td>WMF, NRD</td>
<td>700–900</td>
<td>Y</td>
<td>Y</td>
<td>Quit WMF/At diagnosis</td>
<td>5.5</td>
<td>2.7</td>
<td>1.8</td>
<td>9.1</td>
</tr>
<tr>
<td>9</td>
<td>20/M</td>
<td>FF</td>
<td>500–600</td>
<td>Y</td>
<td>N</td>
<td>Quit formula and encourage solid food/At diagnosis</td>
<td>3.0</td>
<td>1.6</td>
<td>4.4</td>
<td>4.4</td>
</tr>
<tr>
<td>10</td>
<td>27/M</td>
<td>NRD</td>
<td>600–800</td>
<td>Y</td>
<td>Y</td>
<td>Elimination of diary food/ At diagnosis</td>
<td>6.2</td>
<td>2.5</td>
<td>9.2</td>
<td>11.2</td>
</tr>
</tbody>
</table>

CM, cow’s milk; FF, formula feeding, WF, weaning food; BMF, breast milk feeding; WMF, whole milk feeding; AAF, amino acid formula (Neocate®); NRD, normal regular diet; RBC, red blood cell; Hb, hemoglobin; NR, not reported; ND, not done; Y, yes; N, no.

a All patients received oral iron supplements.

b The value corresponds to the volume of goat’s milk-based formula the patient was taking.

c The amount corresponds to the daily intake of dairy products such as cheese which was converted to the equivalent of cow’s milk because the patient did not consume whole cow’s milk or formula.
formula or whole cow’s milk, elimination of dairy food such as cheese or yogurt was recommended.

To analyze the effects of cow’s milk protein on the clinical course, the patients were divided according to the time of complete elimination of cow’s milk-based foods. The early intervention group was composed of five patients and the late intervention group was composed of three patients in which the complete elimination of cow’s milk-based foods was initiated at 0.7–5.1 months after diagnosis because of the unresponsiveness of the IDA to iron supplements and reduction of cow’s milk-based formula or milk intake. After including two patients who underwent no dietary modification into late intervention group, we compared the time of normalization of the laboratory parameters.

Figure 1  Time to normalization of the laboratory parameters according to the dietary intervention (p > 0.05). Early, Early intervention group; Late/No, Late/no intervention group.

Figure 2  Trend of the laboratory parameters with treatment intervention during a 13-month follow-up period in Patient No. 4. CM, cow’s milk; A1AT, α1-antitrypsin.
parameters between early intervention group and late/no intervention group. As a result, the times to normalization of Hb, albumin, and eosinophil count were all shorter in the early intervention group compared to the late/no intervention group, even though the difference was not statistically significant (Fig. 1).

Here, we describe the long-term clinical course of one of the patients in the late dietary intervention group (patient 4 in Table 2). When a 12-month-old boy was diagnosed with PLE and IDA, he was fed with infant formula (total amount of 600 mL per day) along with complementary food. His initial Hb and albumin levels were 5.0 g/dL and 1.8 g/dL, respectively. After albumin infusion at diagnosis without RBC transfusion and initiation of oral iron of 5 mg/kg, his clinical symptoms improved within a few days. His formula intake was reduced to half immediately after the diagnosis while encouraging an increased intake of complementary food. During the 5-month follow-up, the Hb and albumin levels did not normalize, even after increasing the daily dose of oral iron to 20 mg/kg. Additionally, his stool A1AT level increased. After complete removal of formula milk and other dairy foods from his diet at 5.1 months after diagnosis, the normalization of Hb, albumin, and stool A1AT levels was achieved within several weeks. When the cow’s milk-based foods were reintroduced at a later time, there was no clinical or laboratory relapse (Fig. 2).

4. Discussion

To broaden our understanding of the rare condition of PLE combined with severe IDA in infants and toddlers, we presented the clinical characteristics and long-term course of 10 children along with their dietary information and laboratory test results during the long-term follow-up. In this study, complete elimination of formula or whole cow’s milk intake immediately after diagnosis seemed to result in more rapid normalization of the laboratory parameters than a reduction in the amount of formula or whole milk or no dietary change. This finding may provide some clues for determining the disease etiology and treatment strategies.

To date, there have been several case reports of PLE in the setting of mostly severe IDA in infants and toddlers. According to the published cases, the development of the disease is often explained as the consequence of excessive cow’s milk intake even though the mechanism has not been clearly determined. Moreover, as there is no consensus on the treatment strategies, different approaches to dietary management have been suggested, such as complete elimination of cow’s milk or a change to amino acid-based formula, as well as reduction of the amount of cow’s milk intake. In addition, packed RBC transfusions and albumin infusions are also given according to the physician’s preference. Nevertheless, most studies reported clinical improvement shortly after introducing iron supplementation, and the PLE resolved once IDA was corrected.

Considering the nutritional causes of IDA in children, late introduction of iron-fortified complementary food or early introduction of whole cow’s milk before 12 months of age are the main risk factors for ID or IDA in late infancy. Excessive consumption of cow’s milk is another nutritional problem associated with the risk of IDA, especially in toddlers. Regarding the contribution of cow’s milk ingestion to IDA, several mechanisms have been proposed besides reducing the intake of other iron-enriched foods due to excessive cow’s milk consumption. Cow’s milk is one of the most iron-poor foods (0.5 mg/L), and the bioavailability of the iron in cow’s milk is also relatively low compared to human milk. Moreover, the composition of nutrients in cow’s milk, such as relatively high calcium, phosphorus, and casein protein along with relatively low vitamin C, has a negative impact on iron absorption. Cow’s milk ingestion has long been suggested to be associated with GI bleeding, but the association between cow’s milk consumption and GI blood loss has not been clearly explored beyond the first 6 months of age.

In the present study of children aged 7.0–26.7 months, only two patients were fed whole cow’s milk, while most were fed powdered formula. None of the children were exclusively breastfed or started on whole cow’s milk before 1 year of age. Moreover, none of the patients reported the late introduction of complementary foods except for one child with feeding difficulty, suspected of being at a high risk for IDA. According to the dietary guidelines for infants and toddlers to date, the recommended daily volume of cow’s milk intake for children older than 12 months is about 16–24 oz per day (480–720 mL per day). The total amount of cow’s milk or goat’s milk-based formula, or whole cow’s milk ingested by the patients in this study was 300–900 mL per day. Half of the patients on formula feeding did not exceed the recommended maximum volume of 720 mL per day. In particular, the formulas for weaning-age children available in Korean markets have an amount of iron at 6–8 mg/L, which seems to be slightly lower than the recommended iron level of iron-fortified formulas for infants older than 6 months by European and American organizations. However, there is not enough evidence to determine the optimal level of iron fortification for toddlers. Consequently, IDA in this study population could not be fully explained by “excessive cow’s milk consumption.” Therefore, we assumed that there were other causes of IDA besides excessive consumption of cow’s milk among our patients.

Regarding the occurrence of PLE, direct association with cow’s milk has not been clearly demonstrated; rather, IDA associated with cow’s milk seemed to induce PLE. In fact, several studies have concluded that severe IDA resulting from excessive consumption of cow’s milk may consequently lead to an enteropathy. As a result, some authors suggested that oral iron replacement alone could ameliorate this disease entity, regardless of reducing or eliminating cow’s milk intake, and reported that PLE resolved when IDA was corrected. In fact, severe anemia itself directly affects the GI mucosa by impairing epithelial tight junction regulation, thereby increasing mucosal permeability, which results in protein leakage into the lumen of the GI tract, thus leading to PLE. However, since not all children with severe IDA develop PLE, it is conceivable that there may be a systemic process in which both PLE and severe IDA occur simultaneously in susceptible individuals. Early authors have implicated whole cow’s milk as the primary cause of intestinal protein loss, reporting this entity as “allergic gastroenteropathy,” and elimination of
cow’s milk from the diet was suggested as a treatment option. Through IgE, non-IgE-mediated, or mixed-type immunologic reactions, inflammation of GI mucosa may be induced by diverse food proteins, including cow’s milk protein, which often leads to the development of several enteropathies as well as IDA associated with GI blood loss. Some conditions categorized into the non-erosive GI causes of PLE, such as eosinophilic gastroenteritis or food-induced enteropathy, are often associated with food allergens. In fact, the clinical features of PLE with IDA were found in toddlers with cow’s milk ingestion who were ultimately diagnosed with eosinophilic GI disorders after endoscopic biopsies. In our study, peripheral eosinophilia was found in 70% of the patients, while food-specific IgE was not identified in any of the patients except for one; thus, IgE-mediated immunologic reaction was excluded as a possible pathogenesis for this condition. Meanwhile, we could not completely exclude eosinophilic GI disorders in our patients despite the absence of tissue eosinophilia in endoscopic biopsies because of an insufficient number of tissue samples obtained to detect eosinophilic infiltrations, especially from the small bowel.

Cow’s milk protein-induced IDA is known as a severe form of cow’s milk protein allergy (CMPA) that is categorized into the non-IgE-mediated hypersensitivity spectrum in a recent review. In a recent study, CMPA-related IDA was found in 13.7% of the children with IDA aged <4 years. Furthermore, 86% of them demonstrated the clinical features of PLE, and their clinical presentations were consistent with our study subjects, including a high rate of positive stool occult blood and similar endoscopic and histologic findings. The diagnosis of non-IgE mediated food hypersensitivity syndrome is often challenging given the lack of noninvasive confirmatory tests. In practice, most patients are diagnosed clinically based on the history and response to a trial elimination diet and oral food challenge. In a large number of reported cases of PLE with severe IDA, cow’s milk elimination was empirically implemented as treatment, and this trial of food avoidance often resulted in clinical improvement. However, in cases of severe IDA combined with PLE, complete elimination of cow’s milk protein from the diet should be considered immediately at diagnosis, especially when the laboratory parameters are not normalized with an adequate dose of iron supplementation, even though the clinical symptoms improve. A detailed dietary history and appropriate counseling about iron-fortified foods are also recommended during the first year of life to prevent IDA as it is a significant nutritional problem in toddlers.

Declaration of competing interest

The authors declare no conflicts of interest.

References


Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pedneo.2022.08.003.