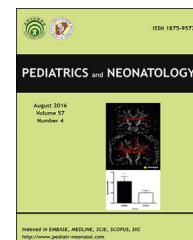


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Original Article

The prevalence and characteristics of cow's milk protein allergy in infants and young children with iron deficiency anemia

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Background: The clinical presentation of cow's milk protein allergy (CMPA) in children varies. This retrospective study aimed to investigate the prevalence and clinical manifestations of CMPA in young children who visited for evaluation of iron deficiency anemia (IDA).

Methods: Patients aged <4 years who were diagnosed as having IDA (serum ferritin <12 ng/mL) at the National Cheng Kung University Hospital, Taiwan in the period 2005–2015 were reviewed. Their clinical presentations, laboratory data, endoscopy findings, and prognosis were analyzed.

Results: Seven of 51 IDA patients (13.7%) had CMPA. The pallor (100%), failure to thrive (43%), and general edema (43%) were the common features. Six (86%) had hypoalbuminemia and four (57%) had positive occult blood in the stool. Of the five patients who underwent skin prick test, four (80%) had positive results. Most of the colonoscopies revealed erosive and hemorrhagic colitis and lymphoid hyperplasia, but none of the biopsies demonstrated eosinophilia in the lamina propria. All of the patients recovered from their IDA within seven months of cow's milk protein elimination and iron supplementation.

Conclusion: CMPA should be considered in young children with undetermined IDA. Cow's milk protein elimination and iron supplementation help in the recovery.

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Abbreviations: CMPA, cow's milk protein allergy; IDA, iron deficiency anemia; EGD, esophagogastroduodenoscopy.

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1. Introduction

Iron deficiency (ID) remains a major cause of anemia in infants and young children. An estimated 0.9–4.4% of toddlers aged 1–3 years old in the United States have iron deficiency anemia (IDA), although the prevalence depends on race/ethnicity and socioeconomic status.¹ Previous studies revealed that inadequate iron intake was a major factor in ID and IDA among children aged <2 years.^{2,3} However, there is a paucity of data on blood loss due to cow's milk protein (CMPA) allergy as a cause of ID or IDA in young children, which is different in Europeans and Asians. Thus, there is a need to evaluate the role of CMPA in young children with undetermined IDA.

The incidence of CMPA in the first year of life is approximately 2–3% in developed countries and accounts for the most common food allergy in early childhood.^{4–6} The symptoms and signs of CMPA vary widely based on the causative mechanism.^{7,8} Immediate reactions include anaphylaxis, acute angioedema, urticaria, and wheezing, whereas late reactions usually present as atopic dermatitis, nonspecific gastrointestinal complaints, including frequent regurgitation, colic, diarrhea with or without intestinal protein or blood loss, and rarely, iron deficiency anemia.⁷ Although IDA may be the sole manifestation of CMPA in infants and children, gastrointestinal mucosal lesions and eosinophilic infiltration are not pathognomonic features of CMPA-related IDA.¹⁰ It is therefore necessary to understand the prevalence and characteristics of CMPA-related IDA in infants and young children.⁹

The present study aimed to assess the features of infants and young children who had CMPA and presented mainly with IDA. This report also provides differential diagnoses when evaluating undetermined IDA in infants and young children.

2. Material and methods

2.1. Patient enrollment

The ICD-10 code D50.9 was used to find patients with a diagnosis of iron deficiency anemia in an 11-year period (between January 2005 and December 2015) at National Cheng Kung University Hospital, a tertiary medical center in southern Taiwan. The hospital's Ethical Committee approved the study protocol (A-ER-105-129). Patients aged 0–4 years, with IDA diagnosis (confirmed by hemoglobin level <11 g/dL and the serum ferritin level <12 ng/mL),^{2,11} and CMPA were included in the study. The diagnosis of CMPA depended mainly on successful result of elimination diet with or without challenge test.

Other factors contributing to iron deficiency, such as insufficient iron intake, malignancy, inflammatory bowel disease, blood loss from other than GI tract, or chronic use of aspirin or nonsteroidal anti-inflammatory drugs, were excluded by focused history-taking, physical examination, and common laboratory tests.

2.2. Methods

The prevalence of CMPA in patients with IDA was calculated. The medical charts of the enrolled patients were carefully reviewed for their demographic data, including the age of disease onset, sex, and dietary history. Aside from pallor and anemia, the common gastrointestinal symptoms and signs of CMPA, such as blood-streaked stool, diarrhea, constipation, emesis, abdominal bloating, abdominal pain, failure to thrive, and general edema were also assessed.

Laboratory data related to cow's milk sensitivity reaction (e.g., determination of specific IgE in blood sample and skin prick test) and findings from upper and/or lower endoscopies were obtained. Lastly, the choice of medical treatment, mainly cow's milk protein (CMP) elimination diet with iron supplementation and/or other medications, as well as outcomes, were evaluated.

2.3. Skin prick test

Some patients underwent skin prick tests with cow's milk extract and egg white allergens (Allermed, Mountain View, CA, USA). Histamine (10 mg/mL) and physiologic saline were the positive and negative control agents, respectively. A wheal ≥ 3 mm was considered positive according to the European Academy of Allergy Asthma and Clinical Immunology (EAACI) guidelines.⁵ All tests were done by the same allergist and the reader of the results was blinded to the clinical manifestations.

2.4. Statistical analysis

The collected data were statistically analyzed using the SPSS 13.0. The data were presented as number (percentage).

3. Results

3.1. Clinical features

There were 7 patients with CMPA from 51 IDA pediatric patients, for a CMPA prevalence of 13.7% in IDA children. Based on their demographic characteristics (Table 1), the patients' median age was 1.4 years (range, 0.5–3.6 years). Three children were younger than one year of age. The male-to-female ratio was 1.3. Six (86%) were on cow's milk based iron-fortified infant formula (>480 mL per day) and one was exclusively breast-fed, although the mother consumed around one liter of raw milk daily. None of them took special formula or partially/extensively hydrolyzed formula.

In terms of the clinical symptoms/signs and laboratory data (Table 2), all of the patients appeared pale and anemic with a mean hemoglobin value of 5.5 g/dL. Failure to thrive and general edema both accounted for 43%. Other nonspecific gastrointestinal symptoms like diarrhea, constipation, bloating, and abdominal pain rarely occurred.

Table 1 Demographic data of the cases of CMPA with IDA (n = 7).

Parameters							
Sex, male:female	4:3						
Median age, years	1.4 [0.5–3.6]						
Diet history (staple food)	N						
Infant formula	6						
Breast milk ^a	1						
Case	1	2	3	4	5	6	7
Body weight, kg	12.2	8.7	9.4	11.8	12.1	8.9	10.9
z-score	0	1	0–0.5	0.5–1	–2	0	–0.5
Body height/length, cm	81.5	68	76	80	94.9	74.6	78.6
z-score	–0.5	0.5	1–1.5	0.5	–1 to –0.5	1–1.5	–1

Abbreviations: *CMPA*, cow's milk protein allergy; *IDA*, iron deficiency anemia.

^a The mother of the exclusively breast-fed infant ingested around one liter of unmodified cow's milk daily.

Moreover, 86% of patients had hypoalbuminemia, defined by serum albumin less than 3.5 g/dL, and 57% were positive for occult blood in stool using guaiac smear method. Peripheral eosinophilia, with eosinophil counts more than 500 cells/ μ L, was noticed in two (29%). None had significantly increased CMP-specific IgE. However, in five patients

who underwent the skin prick test, 4 (80%) were positive. Five patients had received abdominal sonography, but none had ascites accumulation.

3.2. Esophagogastroduodenoscopy and colonoscopy

According to the ESPGHAN guidelines for diagnosis and management of cow's milk protein allergy published in 2012, in patients with otherwise unexplained significant and persistent gastrointestinal symptoms, failure to thrive, or iron-deficiency anemia, upper and/or lower endoscopies with multiple biopsies are appropriate.¹⁰ Four of the patients underwent both esophagogastroduodenoscopy (EGD) and colonoscopy. One received only EGD. Based on the macroscopy results (Table 3), two patients (40%) had erosive and hemorrhagic gastritis, and three (60%) had duodenal lymphoid hyperplasia on EGD. Compared to EGD findings, there were more positive findings on colonoscopy. On colonoscopy, all of the patients had erosive and hemorrhagic colitis, and three (75%) had prominent lymphoid hyperplasia at the terminal ileum and/or colon. Histologic examination revealed that none of them had the typical eosinophilic infiltration of the lamina propria.

3.3. Treatment and prognosis

In terms of treatment and prognosis in CMPA-related IDA (Table 4), six patients were treated with CMP elimination, with some receiving extensively hydrolyzed formula (eHF) administration. For the exclusively breast-fed infant, the mother was advised to avoid all milk and milk product in her diet. The mean duration of elimination diet was executed for 7 months, but varied according to the patients' age and clinical improvement. Iron supplement (5 mg/kg/day) was given to all the patients for 7 months on average because of concomitant iron deficiency anemia. Anti-histamine and prednisolone were given to two and three patients, respectively. In addition, three patients required albumin infusion due to severe edema, while two received blood transfusion on initial presentation.

After the management, those with positive occult blood in their stool were negative after 1.5 months. All recovered from anemia in a mean period of 7 ± 5.59 months, with the follow-up mean hemoglobin 11.7 ± 0.6 g/dL, mean albumin

Table 2 Clinical manifestations and laboratory findings of the cases of CMPA with IDA (n = 7).

Symptoms and signs	N (%), mean	Range, mean
Pallor	7 (100%)	
Blood-streak stool	0 (0%)	
Diarrhea	1 (14%)	
Constipation	1 (14%)	
Emesis	1 (14%)	
Abdominal bloating	1 (14%)	
Abdominal pain	1 (14%)	
Failure to thrive	3 (43%)	
General edema	3 (43%)	
Laboratory data		
Anemia ^a	7 (100%)	
Hb (g/dL)	5.5	3.6–7.4
RBC (10 ⁶ / μ L)	3.18	2.07–4.33
MCV (fl)	59.3	49.4–72.6
Eosinophilia ^b	2 (29%)	
Eosinophil counts (cells/ μ L)	514	174–1326
Hypoalbuminemia ^c	6 (86%)	
Albumin (g/dL)	2.2	1.2–3.5
Positive stool O.B.	4 (57%)	
High serum total IgE ^d	0 (0%)	
Allergy test, positive		
Skin prick test	4 (80%)	
Immuno CAP Specific IgE, to cow's milk	0 (0%)	
Allergen Phadiatop, to cow's milk	0 (0%)	

Abbreviations: *CMPA*, cow's milk protein allergy; *IDA*, iron deficiency anemia.

^a Hemoglobin level <11 g/dL.

^b Eosinophil >500 cells/ μ L.

^c Albumin <3.5 g/dL.

^d Total Immunoglobulin (Ig) E > 100 IU/dL.

Table 3 Macroscopic findings of EGD and colonoscopy in cases of CMPA with IDA (n = 7).

Case	EGD	Colonoscopy
1	Negative	Erosion/hemorrhage
2	Negative	Erosive/hemorrhage with terminal ileum lymphoid hyperplasia
3	Negative	Erosive/hemorrhage with terminal ileum lymphoid hyperplasia
4	Erosion/hemorrhage	ND
5	ND	ND
6	ND	ND
7	Erosive/hemorrhage with duodenal lymphoid hyperplasia	Erosive/hemorrhage with terminal ileum lymphoid hyperplasia
Total no. (%)	2/5 (40%)	4/4 (100%)

Abbreviations: EGD, Esophagogastroduodenoscopy; CMPA, cow's milk protein allergy; IDA, iron deficiency anemia; ND, not done.

4.4 ± 0.16 g/dL, and mean peripheral eosinophil counts 254 ± 300 cells/ μ L.

4. Discussion

This study demonstrates original data for the prevalence and clinical manifestations of CMPA-related IDA in children aged <4 years.

Cow's milk protein allergy (CMPA) is an increasing healthcare concern in infants and young children. It can induce diverse symptoms and involve more than one organ system, including the skin, respiratory tract, and gastrointestinal tract.¹² When involving gastrointestinal tract, there was also a spectrum of different severity, ranging from allergic proctocolitis, and the most common etiologies of rectal bleeding in otherwise healthy young infants, to a more complex condition like food protein-induced enterocolitis syndrome or eosinophilic gastrointestinal disease. In this study, we described a group of infants and young

children with CMPA who sought clinical help initially due to pale-looking.¹³ The phenotype of CMPA is classified into immediate reactions and delayed reactions, depending on the timeline between allergen ingestion and symptoms occurrence or immunologic reaction.^{12,14} Immediate reactions are mostly IgE-mediated and require only minutes up to 2 h after CMP ingestion. In contrast, delayed reactions are usually non-IgE-mediated or mixed mechanism and may take from several hours to even one week to develop symptoms. As such, CMPA presenting with IDA usually involves the non-IgE-mediated delayed type. The symptom onset is late (0.5–3.6 years) compared to the usual gastrointestinal complaints like regurgitation, abdominal colic, anorexia, vomiting, and diarrhea.¹⁵

The prevalence and characteristics of CMPA patients who present mainly with IDA are rarely reported. In this study, no one reported gross blood in stool, which may have resulted from parents' mis-observation, but 57% of them demonstrated positive occult blood in stool detected by guaiac smear method. The mechanisms of iron deficiency in this condition are posited to be due to occult blood loss from gastrointestinal tract because of inflammation, as well as the possible effect of the inhibition of non-heme iron absorption by calcium and casein in cow's milk formula.^{16,17} Also, repeated stool occult blood test may improve the sensitivity of blood loss from gut.

In the present study, the prevalence of CMPA in children younger than 4 years old with IDA is 13.7%. Insufficient iron intake was excluded by focused dietary history in six. One who was exclusively breast-fed had and poor response to iron supplement. Taken together, these indicate that CMPA needs to be considered in cases of pediatric undetermined IDA.

On physical examination, along with pallor, the common symptoms and signs are failure to thrive and general edema. Hypoalbuminemia with general edema is caused by mucosal inflammation and protein-losing enteropathy.^{7,16} Other gastrointestinal complaints are less frequent and mild, and none has skin lesions or respiratory symptoms.

Although CMPA-related IDA is widely believed to be mostly a manifestation of delayed, non-IgE-mediated allergic reaction,^{7,15} four of the patients here were positive for SPT, indicating an immediately IgE-mediated response. Thus, the findings here are consistent with

Table 4 Treatment methods and prognosis of the patients.

Treatment method	N (%)	Mean (SD)
Elimination diet	7 (100%)	
Anti-histamine	2 (29%)	
Prednisolone	3 (43%)	
Iron supplement	7 (100%)	
Albumin infusion	3 (43%)	
Blood transfusion	2 (29%)	
Prognosis		Mean (SD)
Negative stool occult blood test	3 (75%) ^a	
Mean time to recovery, months	1.5	
Recovery from IDA	7 (100%)	
Mean time to recovery, months		7 (5.59)
Follow-up hemoglobin (g/dL)		11.7 (0.6)
Follow-up eosinophil counts (cells/ μ L)		254 (300)
Follow-up albumin (g/dL)		4.5 (0.16)

Abbreviations: SD, standard deviation.

^a One of four patients did not have follow-up of stool occult blood test.

Fiocchi's opinion that a mixed mechanism induced by one allergen can exist in the same patient for the same symptom.¹⁵ Furthermore, it has been pointed out that in patients with positive specific IgE test or SPT a longer period of intolerance can be predicted.¹⁸ Accordingly, this might be the reason why children with CMPA-related IDA had a higher rate of positive SPT in this study.

Colonoscopy, rather than the EGD, could provide more findings on the gut mucosa in this series. On macroscopy, most of the patients demonstrated erosive and hemorrhagic features along with lymphoid hyperplasia.¹⁹ Eosinophils are commonly detected in normal mucosal biopsies from all sites within the gastrointestinal tract where they are dispersed in the lamina propria and, to a lesser extent, in the epithelium. However, the distinction between the upper limit of normal and abnormally increased tissue eosinophils is not well defined. We defined a significant eosinophilic infiltration when eosinophils were found in the lamina propria of gastric antrum >10/HPF, duodenum >20/HPF and colon >20/HPF and when eosinophils were found in the epithelium in more than occasional numbers.^{20,21} However, applying the criteria to our patients, none had the expected eosinophilic infiltration on histologic examination. Recent literature review has shown that macroscopic lesions and histological findings are neither sensitive nor specific for diagnosing CMPA.¹⁰ In terms of treatment, strict avoidance of CMP is currently the main strategy for managing CMPA.^{9,15,22} All the patients here responded to cow's milk protein elimination combined with iron supplementation.

This study has some limitations, including the small patient number and the dependence of CMPA diagnosis on favorable prognosis after allergen elimination. Because we enrolled patients in our hospital according to the ICD-10 code D50.9, cases who had mild-form CMPA and iron deficiency with spontaneous recovery may have been missed. In the current era, the diagnosis of CMPA still needs to be confirmed by allergen elimination and challenge procedure.¹⁵ There is no food challenge procedure in this study because, in the clinical setting, it is time-consuming and relatively unsafe. Furthermore, it is hard to persuade parents to proceed to food challenge test especially after clinical improvement had been achieved after CMP elimination. On the other hand, before diagnosis of CMPA, these patients had received one to two months of iron supplementation but without improvement. The results may exclude inadequate iron intake of these patients. After CMP elimination, the IDA recovered. Taken together, these results strongly suggested CMP-induced IDA. However, without food challenge test, the over-diagnosis of CMPA in children with intractable IDA is still a possibility.

In conclusion, this is the first study to elaborate on the characteristics of children aged <4 years with CMPA who mainly present with IDA. The consideration of CMPA as a differential diagnosis for IDA in this population must be emphasized.

Conflicts of interest

The authors declare that they have no conflicts of interest relevant to this article.

References

1. Baker RD, Greer FR, Committee on Nutrition American Academy of Pediatrics. Diagnosis and prevention of iron deficiency and iron-deficiency anemia in infants and young children (0–3 years of age). *Pediatrics* 2010;126:1040–50.
2. Huang SC, Yang YJ, Cheng CN, Chen JS, Lin CH. The etiology and treatment outcome of iron deficiency and iron deficiency anemia in children. *J Pediatr Hematol Oncol* 2010;32:282–5.
3. Ferrara M, Coppola L, Coppola A, Capozzi L. Iron deficiency in childhood and adolescence: retrospective review. *Hematology* 2006;11:183–6.
4. Rona RJ, Keil T, Summers C, Gislason D, Zuidmeer L, Sodergren E, et al. The prevalence of food allergy: a meta-analysis. *J Allergy Clin Immunol* 2007;120:638–46.
5. Bock SA. Prospective appraisal of complaints of adverse reactions to foods in children during the first 3 years of life. *Pediatrics* 1987;79:683–8.
6. Høst A. Frequency of cow's milk allergy in childhood. *Ann Allergy Asthma Immunol* 2002;89:S33–7.
7. Caffarelli C, Baldi F, Bendandi B, Calzone L, Marani M, Pasquinelli P, et al. Cow's milk protein allergy in children: a practical guide. *Ital J Pediatr* 2010;36:5.
8. Vandenplas Y, Koletzko S, Isolauri E, Hill D, Oranje AP, Brueton M, et al. Guidelines for the diagnosis and management of cow's milk protein allergy in infants. *Arch Dis Child* 2007;92:902–8.
9. Koletzko S, Niggemann B, Arato A, Dias JA, Heuschkel R, Husby S, et al. Diagnostic approach and management of cow's-milk protein allergy in infants and children: ESPGHAN GI committee practical guidelines. *J Pediatr Gastroenterol Nutr* 2012;55:221–9.
10. Nielsen RG, Fenger C, Bindslev-Jensen C, Husby S. Eosinophilia in the upper gastrointestinal tract is not a characteristic feature in cow's milk sensitive gastro-oesophageal reflux disease. Measurement by two methodologies. *J Clin Pathol* 2006;59:89–94.
11. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. *Worldwide prevalence of anemia 1993–2005: WHO global database on anemia*. Geneva: World Health Organization; 2008.
12. Høst A. Cow's milk protein allergy and intolerance in infancy. Some clinical, epidemiological and immunological aspects. *Pediatr Allergy Immunol* 1994;5:S1–36.
13. Feuille E, Nowak-Węgrzyn A. Food protein-induced enterocolitis syndrome, allergic proctocolitis, and enteropathy. *Curr Allergy Asthma Rep* 2015;15:50.
14. Heine RG, Elsayed S, Hosking CS, Hill DJ. Cow's milk allergy in infancy. *Curr Opin Allergy Clin Immunol* 2002;2:217–25.
15. Fiocchi A, Schünemann HJ, Brozek J, Restani P, Beyer K, Troncone R, et al. Diagnosis and rationale for action against cow's milk allergy (DRACMA): a summary report. *J Allergy Clin Immunol* 2010;126:1119–28.
16. Vanderhoof JA, Kleinman RE. Iron requirements for infants with cow milk protein allergy. *J Pediatr* 2015;167:S36–9.
17. Ziegler EE. Consumption of cow's milk as a cause of iron deficiency in infants and toddlers. *Nutr Rev* 2011;69:S37–42.
18. Skripak JM, Matsui EC, Mudd K, Wood RA. The natural history of IgE-mediated cow's milk allergy. *J Allergy Clin Immunol* 2007;120:1172–7.
19. Yu MC, Tsai CL, Yang YJ, Yang SS, Wang LH, Lee CT, et al. Allergic colitis in infants related to cow's milk: clinical characteristics, pathologic changes, and immunologic findings. *Pediatr Neonatol* 2013;54:49–55.
20. Wyllie R, Hyams JS, Kay M. *Pediatric gastrointestinal and liver disease*. 5th ed. United States: Elsevier; 2015.
21. Yantiss RK. Eosinophils in the GI tract: how many is too many and what do they mean? *Mod Pathol* 2015;28:S7–21.
22. Luyt D, Ball H, Makwana N, Green MR, Bravin K, Nasser SM, et al. BSACI guideline for the diagnosis and management of cow's milk allergy. *Clin Exp Allergy* 2014;44:642–72.