

Casein Hydrolysate Formula-Induced Liver Dysfunction in a Neonate With Non-Immunoglobulin E-Mediated Cow's Milk Allergy

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■ Abstract

A 10-day-old male neonate was admitted with bilious vomiting and gross hematochezia. Peripheral eosinophilia, delayed positive skin prick test to artificial milk, and elevated eosinophil cationic protein levels suggested cow's milk allergy. Fluid infusion with prohibition of oral intake improved the digestive symptoms. Breast-feeding was resumed on hospital day 3 and only casein hydrolysate formula was fed from day 7 onward. Nevertheless, eosinophilia and elevated transaminase levels developed on day 14. Liver dysfunction associated with casein hydrolysate formula was suspected and the infant was transferred to soy formula. Eosinophil counts decreased and transaminase levels were normalized on day 19. A cow's milk protein-specific lymphocyte proliferation test was positive for γ -casein, β -lactoglobulin, and bovine serum albumin, indicating sensitization of T cells to cow's milk proteins. These observations suggest that careful attention should be paid to liver dysfunction in non-immunoglobulin E-mediated cow's milk allergy, even when hypoallergenic formula is used.

Key words: Casein hydrolysate formula. Liver dysfunction. Neonate. Milk allergy.

■ Resumen

Un neonato varón con diez días de vida ingresó con vómitos biliares y rectorragia considerable. La eosinofilia periférica, el resultado positivo de la prueba cutánea retardada para la leche artificial y los niveles de proteína catiónica del eosinófilo elevados, sugirieron una alergia a la leche de vaca. La administración de nutrición parenteral con prohibición de ingesta oral mejoró los síntomas digestivos. La lactancia materna se reanudó en el hospital al tercer día y a partir del séptimo día sólo se le alimentó a base de una fórmula de caseína hidrolizada. No obstante, el día 14 aparecieron la eosinofilia y las elevadas concentraciones de transaminasa. Se sospechó que la disfunción hepática podía estar asociada a la fórmula de caseína hidrolizada, por lo que se pasó a alimentar al lactante con una fórmula de soja. Las concentraciones de eosinófilos disminuyeron y las de transaminasa se normalizaron el día 19. La prueba de proliferación de linfocitos específica de las proteínas de la leche de vaca resultó positiva para γ -caseína, β -lactoglobulina y seroalbúmina bovina, indicando que existía una sensibilización de las células T a las proteínas de la leche de vaca. Estas observaciones sugieren que hay que prestar especial atención a la disfunción hepática en los casos de alergia a la leche de vaca no mediada por la inmunoglobulina E, incluso cuando se emplean fórmulas hipoalergénicas.

Palabras clave: Fórmula de caseína hidrolizada. Disfunción hepática. Neonato. Alergia a la leche.

Introduction

Accumulated research findings since the first report of allergic intestinal bleeding by Rubin [1] in 1940 have revealed that cow's milk allergy is the immune response to 1 or more of the proteins in milk [2]. Cow's milk is one of the most frequent causes of food allergy [3]. When milk allergy develops in the neonatal period, gastrointestinal manifestations such as hematochezia, diarrhea, abdominal distension and vomiting are frequently exhibited. Since gastrointestinal disease requiring surgical intervention may be suspected on the basis of such symptoms, it is often difficult to make an accurate diagnosis of cow's milk allergy.

Recently, infant cases of liver dysfunction complicated by cow's milk allergy have been reported to be improved by elimination of cow's milk formula [4,5]. In this report, we describe a neonate with non-immunoglobulin (Ig) E-mediated cow's milk allergy who developed marked eosinophilia and liver dysfunction associated with casein hydrolysate formula.

Case Description

A 10-day-old male neonate was referred to Nara Medical University Hospital with bilious vomiting and gross hematochezia that had developed suddenly on the preceding day. There was no maternal or birth history of interest. The neonate was the mother's first child and was born at 38 weeks gestation by spontaneous delivery, with a birthweight of 2958 g. He had taken supplementary feeding of breast milk since just after birth. His father had no history of allergic diseases but his mother had a pollen allergy. Six days after birth, he was discharged from hospital weighing 2910 g.

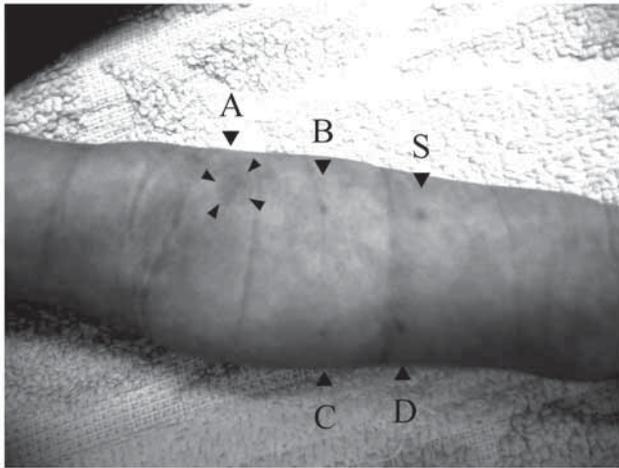


Figure 1. Results of skin prick tests 3 hours after the needle prick. (A) artificial milk (Hohoemi infant formula, Meiji Dairies, Tokyo, Japan); (B) breast milk; (S) saline; (C) casein hydrolysate formula (MA-1, Morinaga Milk Industry, Tokyo Japan); (D) whey-casein hydrolysate formula (MA-mi, Morinaga Milk Industry). The artificial milk and formulae were prepared ready for feeding. Skin prick test was positive for artificial milk (8 × 7 mm) but negative for breast milk and the other formulae.

When the infant was admitted to our facility, he had a body temperature of 37.4°C and a body weight of 2702 g, indicating weight loss. His anterior fontanelle was soft and flat, and skin turgor was normal. The palpebral conjunctiva was not anemic. His heartbeat was regular without murmurs, and lung sounds were clear. The abdomen was soft, flat, and nontender, with active bowel sounds. Hepatosplenomegaly was not observed. An X-ray examination of the abdomen revealed decreased intestinal gas, but a radiographic contrast study of the upper and lower gastrointestinal tract was unremarkable. Fecal eosinophilia was not observed. The infant had increased white blood cell counts of 14400 cells/L with eosinophilia (2160 cells/L). The platelet count was $409 \times 10^3/L$ and hemoglobin 12.5 g/dL. Hemostatic tests such as prothrombin time, activated partial thromboplastin time, and bleeding time were normal. Blood biochemistry showed hypoproteinemia: total protein of 4.0 g/dL and serum albumin of 2.8 g/dL. Elevated transaminase levels were not observed. C-reactive protein was negative. Bacteriological culture from the feces was negative. In allergy tests, total serum IgE was below the detection limits. In addition, specific IgE antibodies to whole cow's milk, -lactalbumin, -lactoglobulin, and casein were all negative by fluorescent enzyme immunoassay (CAP-FEIA; Pharmacia Diagnostic, Uppsala, Sweden).

Skin prick test (SPT) was performed with artificial milk (Hohoemi Infant Formula, Meiji Dairies, Tokyo, Japan), breast milk, saline, casein hydrolysate formula (MA-1, Morinaga Milk Industry, Tokyo, Japan), and whey-casein hydrolysate formula (MA-mi, Morinaga Milk Industry). SPT was initially negative for the artificial milk that the infant had received, but a positive flare developed 3 hours later. However, the test was negative for breast milk, casein hydrolysate formula, and whey-casein hydrolysate formula (Figure 1). Furthermore, the concentration of eosinophil cationic protein (ECP) increased to more than 150 g/L. The clinical course is shown in Figure 2.

Neonatal bleeding tendency was initially suspected but was later ruled out based on laboratory findings showing normal hemostatic function without anemia. Secondly, gastrointestinal diseases requiring surgical intervention such as malrotation of the intestine were suspected, but were ruled out by routine workups. Peripheral eosinophilia led us to suspect cow's milk allergy. Fluid infusion was commenced with prohibition of oral intake, and this improved digestive symptoms on the following day. Based on the results of SPT, breast-feeding was resumed on hospital day 3.

Casein hydrolysate formula was added as a substitute for breast milk on hospital day 5, and only the casein hydrolysate formula was fed from hospital day 7 onward. Suckling behavior improved substantially, vomiting and hematochezia were resolved, and favorable recovery of body weight was observed. On day 14, however, eosinophil counts increased further to 11 822 cells/L, aspartate aminotransferase (AST) to 247 IU/L, alanine aminotransferase (ALT) to 140 IU/L, -GTP to 263 IU/L, and direct bilirubin to 0.7 mg/dL, indicating liver damage [6]. Hepatitis B surface antigen, antibodies for hepatitis A virus, hepatitis C virus, cytomegalovirus, and herpes simplex virus, and IgM antibody to Epstein-Barr viral capsid antigen were all found to be negative. Ultrasound examinations

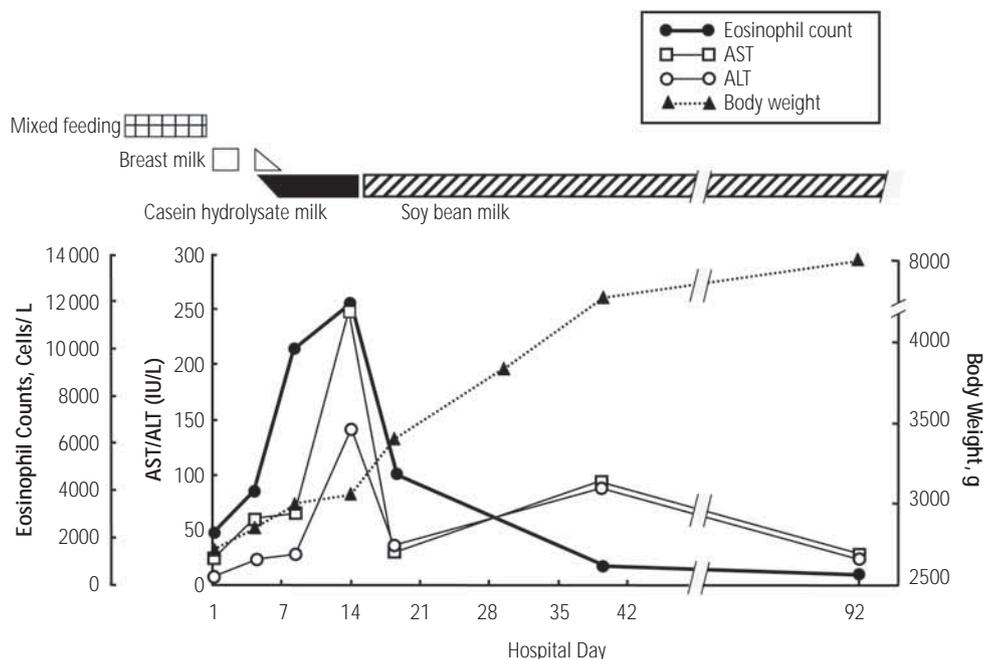


Figure 2. Clinical course. AST indicates aspartate aminotransferase; ALT, alanine aminotransferase.

of the liver showed a normal appearance. Liver dysfunction associated with casein hydrolysate formula was suspected, and the formula was changed to an amino acid formula. Since the patient would not take the formula, however, we were compelled to change it to soy formula (Bonlact, Wakodo, Tokyo, Japan), which has been reported to be nutritionally equivalent to hydrolyzed milk formula [7]. Five days after the shift to soy formula (hospital day 19), eosinophil counts decreased to 4624 cells/L, and transaminase levels were normalized (AST, 29 IU/L; ALT, 35 IU/L).

The infant was discharged without any complications. Pending substantial recovery of the liver, a cow's milk protein-specific lymphocyte proliferation test [4] was performed at 7 weeks old. The percentage of the S-phase cells after milk protein stimulation was converted into a stimulation index using the following equation and expressed as SIF (stimulation index measured by flow cytometry):

$$\text{SIF} = (\text{S-phase cells (\%)} \text{ in a stimulated sample} / \text{S-phase cells (\%)} \text{ in an unstimulated sample}) \times 100$$

The SIF was 574 for -casein (mean [SD] in controls, 250.3 [58.3]; n=11), 369 for -lactoglobulin (control, 110.4 [30.6]; n=10), and 376 for bovine serum albumin (control, 120.5 [27.2]; n=8), indicating T-cell sensitization to cow's milk proteins. CAP-FEIA did not reveal the presence of cow's milk-specific IgE at this age.

The blood test at 6 months old showed normal eosinophil counts (320 cells/L), AST (38 IU/L), ALT (35 IU/L), -GTP (14 IU/L), and serum ECP (4.6 g/L). Specific IgE to cow's milk, casein, and soybean were less than 0.34 kU/L, whereas IgE for egg albumen was 3.11 kU/L (measured by CAP-FEIA). To date, no severe allergic reactions have occurred since the patient was discharged. The patient has achieved normal growth and development with a diet lacking cow's milk and eggs.

Discussion

There is currently no widely-accepted specific method for diagnosis of non-IgE-mediated cow's milk allergy. Neonatal surgical diseases will be primarily suspected in the case of digestive symptoms [8], which are often severe [9]. Therefore, invasive tests such as Meckel's diverticulum scintigraphy, radiographic contrast enema, and upper gastrointestinal series will be performed for differential diagnosis. Earlier diagnosis of non-IgE-mediated cow's milk allergy is desirable to avoid unnecessary invasive testing. It would be practically difficult to perform oral food challenge testing, however, especially in a neonate. Therefore, we must often make the diagnosis of non-IgE-mediated cow's milk allergy based on assessment of clinical manifestations and laboratory analysis. Results of laboratory tests, including eosinophil counts in peripheral blood or fecal mucus, SPT, and rectal biopsy, as well as analysis of total and specific IgE, should be interpreted with caution. Most recently, however, the usefulness of an allergen-stimulated peripheral lymphocyte proliferation test in the diagnosis of non-IgE-mediated cow's milk allergy has been reported [4].

In our case, SPT was positive for whole cow's milk formula with a delay of 3 hours. This late-phase response suggested the presence of an IgE-mediated cow's milk allergy. However, laboratory data suggested the involvement of a cellular immune response; the eosinophil count was as high as 2160 cells/L on admission, but neither total IgE nor cow's milk protein-related IgE antibody was detected. ECP [10,11] was markedly increased. Furthermore, in the allergen-stimulated peripheral lymphocyte proliferation test, the patient was positive for -casein, -lactoglobulin, and bovine serum albumin. Taken together, these findings indicate that a cellular immune response, including the sensitization of

T cells to cow's milk proteins, would play a major role in the underlying mechanism.

Elevated transaminase levels due to casein hydrolysate formula or amino acid formula have been reported [12], but the underlying mechanism remains unknown. Saito et al [4] examined infants with non-IgE-mediated cow's milk allergy who showed elevated transaminase levels without extrahepatic symptoms, which are improved by eliminating cow's milk formula. In that study, cow's milk protein-specific lymphocyte proliferation tests were positive, although total and milk protein-specific IgE antibody was not detected in such cases.

In our case, although casein hydrolysate formula drastically improved the digestive symptoms, hypoproteinemia, abnormal body weight gain, and liver dysfunction developed with further increased eosinophil counts. Elimination of casein hydrolysate formula led to the reduction of eosinophil counts and the normalization of transaminase levels, suggesting association of a cellular immune response to casein hydrolysate with liver dysfunction. Since immunopotency of hydrolyzed cow's milk formula has been demonstrated [13], the pathogenic mechanism of such liver dysfunction might be similar to that of non-IgE-mediated, allergic forms of drug-induced liver injuries [14,15]. However, further studies will be required to address this issue.

High molecular weight proteins as large as 10 to 70 kd are absorbed from the gastrointestinal tract and effectively presented as antigens by antigen presenting cells. Milk allergy formula achieves low antigenicity by hydrolytic degradation of casein or whey in milk constituent to less than 1 kd. Since extensively hydrolyzed protein-intolerant patients show a higher frequency of persistent and multiple food intolerance than patients tolerating casein hydrolysate [16], we should carefully consider the possibility of multiple food allergies in the patient. Although there was concern over the possibility that long-term soy protein feeding might provoke an allergic reaction, no severe allergic episodes have since emerged.

In conclusion, this case suggests that when hypoallergenic formulae such as casein hydrolysate formula are used in non-IgE-mediated cow's milk allergy, we should pay careful attention to liver dysfunction as well as hypoproteinemia, even if digestive symptoms are not present.

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