

## Milk allergy in the neonatal intensive care unit: comparison between premature and full-term neonates

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**Background:** There have been several reports on neonates with milk allergy in a neonatal ward. This type of allergy is mostly categorized as a non-IgE-mediated food allergy. Although most cases of milk allergy occur in the first few months of life, the differences in clinical characteristics between premature and full-term neonates are still unclear.

**Objective:** This study aimed to clarify the differences in clinical characteristics of milk allergy between premature and full-term neonates.

**Methods:** We retrospectively evaluated 2,116 neonates admitted to the Department of Neonatology, Chiba Kaihin Municipal Hospital, between 2001 and 2007.

**Results:** We identified 24 neonates strongly suspected of having-milk allergy because of symptoms, such as bloody stools, repeated vomiting, diminished sucking and abdominal distension, as well as objective laboratory findings of eosinophilia in stool cytology and/or positive results for a rectal milk challenge test. Twelve of these 24 neonates were premature (median gestational age,  $31 \pm 3$  weeks; median birth weight,  $1,656 \pm 592$  g) and the other 12 were full-term (median gestational age,  $38 \pm 1$  weeks; median birth weight,  $2,760 \pm 560$  g). There were no differences in symptoms and time to start of feeding between premature and full-term neonates, but there was a significant difference in the median postnatal age at onset (premature neonates: 23 days; vs. full-term neonates: 3.5 days;  $p < 0.01$ ).

**Conclusion:** All premature neonates developed a milk allergy after 32 weeks of corrected gestational age, suggesting that the development of milk allergy requires a certain degree of immunological maturation.

**Key words:** Milk allergy; Neonates; Prematurity; Term; Immune maturation; Onset

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## INTRODUCTION

Most neonatal milk allergies are non-IgE-mediated and are classified according to symptoms, into food protein-induced proctocolitis syndrome, food protein-induced enterocolitis syndrome (FPIES), and food protein-induced enteropathy [1-8], or are assessed by cluster analysis according to clinical and laboratory findings [9]. Milk allergy in neonates is currently diagnosed based on medical history, clinical features, recovery from symptoms by removing casual allergens, and an oral food challenge test (OFC) [8, 10]. However, in clinical settings, it is often difficult to distinguish milk allergy in neonates from sepsis, shock, or surgical diseases [11-13], because neonatal patients with milk allergy sometimes present with nonspecific symptoms, such as fever and abdominal distension, and accurate laboratory tests have not been established for non-IgE-mediated allergies.

Although milk allergy in neonates is an important disease in neonatal medicine, its prevalence among neonates remains unclear. A multicenter survey conducted using questionnaires in Japan showed a 0.21% prevalence of milk allergy in neonates in Japan [14]. However, the institutions in this multicenter survey did not use unified criteria for milk allergy. Although a survey using unified criteria was conducted for FPIES in a patient series in a children's hospital in Australia [15], infants other than neonates were also included in this survey (mean age, 5.5 months). In addition, there have been no patient series for milk allergy or FPIES among neonates comparing premature and full-term neonates using unified criteria in one hospital, and little is known regarding whether there are differences in clinical and laboratory features between these neonates with milk allergy.

Therefore, this study aimed to estimate the prevalence of milk allergy in neonates and clarify the differences in clinical characteristics of milk allergy between premature and full-term neonates. We conducted a retrospective study of neonates admitted to the Department of Neonatology, Chiba Kaihin Municipal Hospital.

## MATERIALS AND METHODS

### Data collection

We conducted a retrospective study of neonates admitted to the Department of Neonatology, Chiba Kaihin Municipal Hospital, between 2001 and 2007. This study was approved by

the ethics committee. First, all admitted patients were screened for suspected milk allergy based on international classification of diseases codes. We then checked the clinical records of patients suspected of having milk allergy and collected the following information: gestational age, birth weight, sex, family history of allergy, time to start of feeding, type of feeding, symptoms, postnatal age at onset, preexisting disease, results of stool cytology and a rectal milk challenge test [16], eosinophil count in peripheral blood, cow's milk-specific IgE using the ImmunoCap (Phadia, Sweden), and treatment.

### Feeding

When a patient received cow's milk-based formula once, we regarded the type of feeding as mixed feeding. For premature patients, we regarded the day of starting regular feeding as the "time to start of feeding." Intraoral application was not included. Both premature and full-term patients were either fed with human milk only, mixed feeding, or cow's milk only.

### Diagnosis

Patients who presented only with fecal occult blood or a small amount of bloody stools were suspected of allergic proctocolitis and were excluded in this retrospective study because of its mild non-systemic symptoms. Patients who presented with several symptoms, such as bloody stools, vomiting, diminished sucking, and abdominal distension, were suspected of having allergic enterocolitis. Among the suspected cases of allergic enterocolitis, we diagnosed patients with milk allergy when they had objective findings, such as eosinophilia in stool cytology (positive criteria: seen more than 10 eosinophils under 500 times magnification in 5 fields), a positive response to a rectal milk challenge test, and resolution of symptoms after changing their diet to fully-hydrolyzed formula. Although milk allergy is generally diagnosed by an OFC, we did not perform an OFC for all neonates because of its risks. Instead of OFC, we did rectal challenge test [16]. When eosinophils in stool increased beyond the positive criteria we judged it is challenge test-positive.

### Statistical analysis

Statistical analysis was performed by comparing median values using the Mann-Whitney U test and Spearman's rank correlation coefficient using SPSS ver.11.0.1 J (SPSS Inc., USA).

## RESULTS

### Prevalence of milk allergy in the neonatal ward

A total of 2,116 neonates (1,208 males, 905 females; mean gestational age =  $35 \pm 4$  weeks; mean birth weight =  $2,137 \pm 765$  g) were admitted to the Department of Neonatology, Chiba Kaihin Municipal Hospital, between 2001 and 2007. Of 61 babies suspected of having milk allergy, 18 were excluded because they were diagnosed with allergic proctocolitis. Of 43 neonates that presented with symptoms, such as bloody stools, vomiting, diminished suckling ability, and abdominal distension, 24 had various laboratory features, such as eosinophilia in stool cytology and/or positive results for a rectal milk challenge test; in addition, their symptoms improved on a hydrolyzed formula. We diagnosed these 24 neonates as having a milk allergy (1.13%). There were no differences in sex, birth weight and gestational age between milk allergic patients and all patients on admission (data not shown).

### Characteristics of premature and full-term milk allergic patients

With regard to maturity, 12 milk allergic patients were premature (7 males, 5 females) and 12 were full-term neonates (7 males, 5 females). Their characteristics are shown in Table 1. Seven premature patients were low birth weight infants, 3 were very low birth weight infants, and 2 were extremely low birth weight infants. In addition, there was a pair of dichorionic diamniotic twins among the premature patients. There were no differences in allergic family history between the premature and full-term patients. There were no differences in the feeding patterns between the 2 groups. "Time to start of feeding" was a median of 1 day (range: 0-7 days) in both premature and full-term patients, with no difference between the 2 groups.

Full-term patients had some preexisting diseases, which was why they were admitted to the Department of Neonatology. Five had transient tachypnea of newborns, 4 had neonatal jaundice, and 1 suffered from neonatal convulsions. These 10 neonates developed milk allergy after admission. Only 2 neonates were admitted because of milk allergy.

### Symptoms of premature and full-term milk allergic patients

Gastrointestinal symptoms were observed most frequently, such as vomiting or bloody stools. Nonspecific symptoms, such as apnea and cyanosis, were present in some cases (Table 1).

Although biliary vomiting was more frequent in premature patients than in full-term patients, there was no significant difference between the 2 groups ( $p = 0.16$ ).

### Laboratory features of full-term and premature milk allergic patients

Laboratory features, such as eosinophil counts in peripheral blood, milk-specific IgE antibody, and positive C-reactive protein, are shown in Table 1. There were no differences in laboratory findings between premature and full-term patients. There were some cases where both premature and full-term patients presented with severe eosinophilia in peripheral blood. Only 1 patient who was premature and 2 patients who were full-term were positive for milk-specific IgE (positive,  $>0.7U_A/mL$ ).

### Postnatal age at onset

Postnatal age at onset was significantly higher in premature patients (median, 23 days; range, 4-49 days) than in full-term patients (median, 3.5 days; range, 1-16 days;  $p < 0.01$ , Fig. 1). In addition, there was a significant negative correlation between postnatal age at onset and gestational age for premature patients (Spearman  $r = -0.65$ ,  $p < 0.05$ , Fig. 2A). Premature patients developed milk allergy after 32 weeks of corrected gestational age (Fig. 2B). There was no significant correlation between the postnatal age at onset and the days to "time to start of feeding" ( $p = 0.37$ ).

## DISCUSSION

To the best of our knowledge, this is the first study on the differences in clinical characteristics between premature and full-term milk allergic patients. In this study, the incidence of milk allergy with systemic symptoms in a neonatal ward was 1.1%. This is higher than that found in a recent multicenter clinical questionnaire-based survey in Japan regarding neonatal milk allergy by Miyazawa et al. [14] (0.21%) in Japan. In their survey, the diagnosis of milk allergy was based on the criteria used at each institution, which were not unified. In addition, respondents might have only reported severe and typical cases of milk allergy in their questionnaires. In contrast, our study might include mild cases. These reasons may explain the differences in the prevalence of milk allergy between our study and that by Miyazawa et al. [14].

In our study, there was a significant delay in onset on milk

**Table 1.** Comparison of premature and full term milk allergic patients

Clinical characteristics	Premature patients (less than 37 weeks gestation) N = 12	Full term patients (37 to 42 weeks gestation) N = 12
<b>Demographics</b>		
Male/female (n)	7/5	7/5
Birth weight (g)	1,589 (662-2,460)*	2,920 (1,886-3,936)*
Gestational age (weeks)	31 (25-36)	38 (37-41)
Family allergic history		
Paternal allergic disease (n)	4	7
Maternal allergic disease (n)	4	3
Feeding pattern		
Human milk only (n)	3	1
Mixed feeding (n)	4	7
Cow's milk only (n)	5	4
<b>Symptoms</b>		
Vomiting	9	8
Biliary vomiting	5	1
Bloody vomiting	0	1
Bloody stools	8	7
Less vigorous sucking	1	4
Abdominal distension	1	1
Gastric hemorrhage	1	0
Toxicoderma	0	1
Cyanosis	0	1
Ileus	0	1
Apnea	1	0
Ill temper	0	1
<b>Laboratory data</b>		
Count of eosinophil in peripheral blood	1,100 (174.5–5,626)*	885 (501.3–1,169)*
Milk specific IgE antibody: positive (>0.7U <sub>A</sub> /mL), (n/total)	9.0% (1/11)	16.7% (2/12)
C-reactive protein: positive (>0.5 mg/dL), (n/total)	8.3% (1/12)	8.3% (1/12)
Stool cytology: eosinophilia positive, (n/total)	100% (9/9)	87.5% (7/8)
Rectal milk challenge test: positive, (n/total)	100% (5/5)	100% (7/7)

\*Median (range).

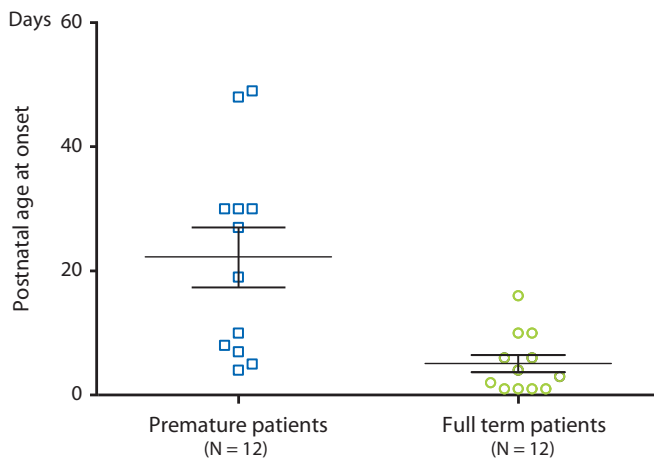
allergy in premature patients compare to full term patients. There are 2 possibilities that might explain the difference in postnatal age between the 2 groups.

First, the amount of milk given to premature neonates was too small to induce allergic reactions to cow's milk. Because of a very small amount of antigen to induce symptoms, premature neonates may require more time before presenting with symptoms compared with full-term neonates, or they may develop T cell

sensitization. However, in some cases, neonates present with symptoms with a small amount of antigen [17]. Although the amount of milk per body weight was different for each neonate, all of them presented with symptoms of milk allergy after 32 weeks of corrected gestational age. Therefore, we assume that the amount of milk given to a neonate is not a major reason for the difference in onset time of allergic symptoms.

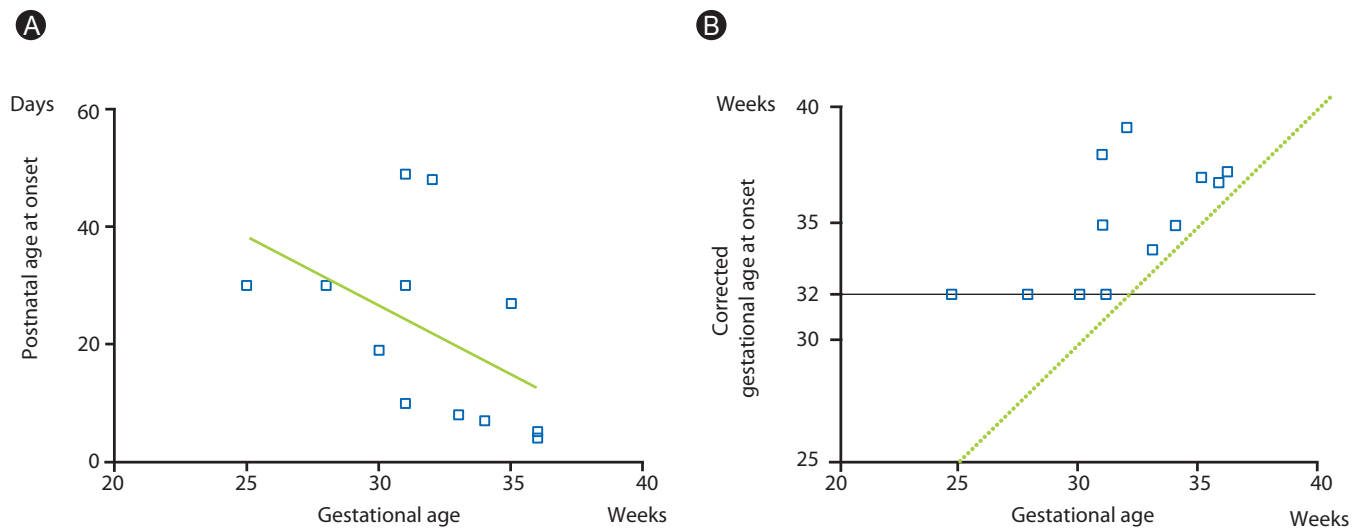
Second, immune immaturity in premature neonates might

explain the difference in postnatal age between the 2 groups. Some reports have shown a correlation between gestational age and cell-mediated immunity. Jones et al. [18] showed that the proportion of proliferating peripheral blood mononuclear cells was different based on gestational age; there was an increase in the proportion of proliferating cells, and this was correlated with increasing gestational age following stimulation



**Fig. 1.** Postnatal age at onset of premature and full-term milk allergic patients. Premature milk allergic patients had a significantly higher postnatal age at onset than full-term milk allergic patients ( $13.6 \pm 14.5$  days for premature milk allergic patients [N = 12] and  $5.1 \pm 4.8$  days for full-term milk allergic patients [N = 12]; Mann-Whitney U test;  $p < 0.01$ ).

with phytohemagglutinin or  $\beta$ -lactoglobulin. Warner et al. [19] reported a positive correlation between the percentage of T cells in umbilical cord blood expressing the marker CD45RO and gestational age. Therefore, at an early gestational age, cell-mediated immunity is thought to be more immune-immature. Because food protein-activated intestinal lymphocytes are thought to be important in the development of milk allergy by elaborating inflammatory cytokines [7, 11, 20], these results suggest that premature neonates require immune maturation prior to the onset of milk allergy. All of premature neonates in the present study had onset of milk allergy after 32 weeks of corrected gestational age. Therefore, the development of milk allergy may require immune-maturation matching at approximately 32 weeks of corrected gestational age. So far, a few studies have shown corrected gestational age when symptoms of milk allergy were present. Dupont et al. [21] investigated milk allergy in a premature patient series. They showed the potential of the atopy patch test as a diagnostic tool in milk allergy in 14 premature patients who were born at  $31 \pm 2$  (mean  $\pm$  SD) weeks of gestation, and in their study, patients developed milk allergy at the age of  $42 \pm 18$  days old. The onset age of milk allergy in this previous study is consistent with our results. Among a few reported milk allergy cases in premature neonates [17, 22], Srinivasan et al. [22] reported that preterm neonates with allergic enterocolitis manifested as recurrent necrotizing enterocolitis (NEC) before 32 weeks. In their



**Fig. 2.** Effect of gestational age on time of onset in premature milk allergic patients. □ indicates each of premature milk allergic patients. (A) Relation between postnatal age at onset of milk allergy and gestational age. There was a significant negative correlation between postnatal age at onset of milk allergy and gestational age for premature patients (Spearman  $r = -0.65$ ;  $p < 0.05$ ). (B) Age at onset of milk allergy by corrected gestational age and gestational age. Premature patients developed milk allergy after 32 weeks of corrected gestational age.

report, NEC with a high eosinophil count showed a trend towards a later time of onset compared with NEC without presentation of systemic eosinophilia [23], suggesting that a Th2-type adaptive immune response requires immune maturity. Therefore, we postulate that premature neonates are not prone to developing milk allergy until maturation of their immune system, which occurs at around 30-32 weeks of corrected gestational age.

In this study, there were four milk allergic neonates fed with human milk only. There are some cases that presented the symptom of milk allergy by exclusively breast-feeding are reported [9, 24, 25]. We consider that the milk protein passed into the breast milk and it caused symptom, like these cases.

This study has several limitations. First, this study was retrospective but not prospective. Second, although the OFC test is generally recommended for diagnosing milk allergy, we did not perform OFC at the early stage of milk allergy but used rectal challenge test. Nevertheless, we believe that our study findings have important implications, because they show the prevalence of milk allergy in the neonatal ward and the different clinical characteristics of premature and full-term milk allergic patients. Our results also suggest that immune maturity is required for the development of milk allergy in neonates. Additional studies are needed to evaluate cell-mediated immune responses in neonates with milk allergy.

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