

The Most Common Cow's Milk Allergenic Proteins with Respect to Allergic Symptoms in Iranian Patients

Raheleh Shokouhi Shoormasti¹, Mohammad Reza Fazlollahi¹, Saeedeh Barzegar¹, Pegah Teymourpour¹, Zahra Yazdanyar¹, Zahra Lebaschi¹, Maryam Nourizadeh¹, Behnaz Tazesh¹, Masoud Movahedi², Homa Kashani³, Zahra Pourpak¹, and Mostafa Moin^{1,2}

¹ Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran

² Department of Immunology and Allergy, Children Medical Center, Tehran University of Medical Sciences, Tehran, Iran

³ Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Received: 12 May 2015; Received in revised form: 5 July 2015; Accepted: 28 August 2015

ABSTRACT

Cow's milk allergy (CMA) is an immunological response to cow's milk proteins such as casein, α -lactalbumin and β lactoglobulin. The aim of this study was to determine the most common cow's milk allergenic proteins in patients with CMA and identify the most effective proteins in different allergic symptoms.

Eighty seven patients (≤ 18 years) with allergy to cow's milk from 2006 to 2013 entered this study. They had a positive history of allergic reactions to cow's milk and a positive specific IgE test to whole cow's milk. The patients' symptoms were divided into four groups. Serum specific IgEs against four different main proteins of cow's milk were measured using RIDA Allergy Screen.

Among 87 patients, 53 (60.5%) were male and the median age was 2.5 years. The frequency of respiratory, skin, gastrointestinal symptoms, and anaphylaxis were 63.3%, 55.7%, 20.3%, and 13.4%, respectively. Specific IgEs to total cow's milk protein ($n=75$, 89.3%), and the main Cow's Milk Proteins including α -lactalbumin ($n=65$, 77.4%), casein ($n=64$, 75.3%), β -lactoglobulin ($n=52$, 62.7%), and bovine serum albumin ($n=35$, 44.9%) were detected. Specific IgE tests to β -lactoglobulin were positive in 90% of the patients with anaphylaxis. Moreover, significant relationship was found between specific IgE to β -lactoglobulin and anaphylaxis ($p=0.04$).

Although it is presumed that α -lactalbumin and casein are the most common allergenic proteins of cow's milk, in this study there is a significant relationship between the anaphylaxis and the presence of β -lactoglobulin-specific IgE. Therefore, more precautions are recommended due to possible anaphylactic reactions in patients with a positive test history for β -lactoglobulin specific IgE.

Keywords: α -lactalbumin; β -lactoglobulin; Bovine serum albumin; Casein; Cow's milk allergy; Cow's milk proteins

INTRODUCTION

Cow's milk protein allergy (CMPA), as one of the most common food allergies, involves 2 to 6% of children, especially during the first year of life.^{1,2} Cow's milk is also the most common food allergen in developing the food allergy and anaphylaxis in Iranian children.³ Although half of these patients recover in the second year of life, it is necessary to precisely identify the cow's milk allergy (CMA) in patients to prevent the low bone density, anemia, and failure to thrive.¹ Although IgG and IgA are the most abundant types of immunoglobulins produced against food antigens but high level of specific IgE is the main cause of allergies.⁴ Anaphylactic reactions to cow's milk protein (CMP) are the most severe symptoms in CMA, however a wide spectrum of manifestations are considered for CMA⁵ including (eczema, asthma and/ or/ rhinitis, nausea and diarrhea).^{6,7}

There are more than 40 types of proteins in cow's milk which have antigens similar to human species.⁸ These proteins have different origins. The most important proteins that the cow's mammary glands produce include whey proteins (α -lactalbumin, β -lactoglobulin) and caseins. Besides, some other proteins such as bovine serum albumin (BSA), lactoferrin or immunoglobulins (Igs) originate from the blood.⁹

Allergenicity of cow's milk proteins in children with CMA has been shown in different studies worldwide,¹⁰ it is for the first time reporting the most common cow's milk allergen with respect to allergic symptoms in Iran. Although the gold standard of diagnostic test for food allergies is the food challenge test but evaluation of specific IgE to CMPs (accompanied by allergic symptoms) can also be a useful test in diagnosis of cow's milk allergy.¹¹ Thus it would be applicable for preventing the possible anaphylactic reactions caused by an oral food challenges.¹⁰

The aim of this study was to determine the most common cow's milk allergenic proteins in patients referred to Immunology, Asthma and Allergy Research Institute (IAARI) with CMA and, to identify the most effective proteins in different allergic symptoms.

MATERIALS AND METHODS

All patients (n=87, ≤ 18 years) with a positive history of CMA and specific IgE levels ≥ 0.35 IU/ml against

whole Cow's milk proteins (CMPs) who had been referred to Immunology, Asthma and Allergy Research Institute (Tehran, Iran) between Jan 2006 and Dec 2013 were entered this study. A specific questionnaire including demographic characteristics, clinical manifestations and principal complaints was fulfilled for them. Two milliliters whole blood was collected after obtaining the informed consent from each patient or their parents and the sera were stored at -70°C . The patients' symptoms were divided into respiratory symptoms (coughing, breathlessness, wheezing, and rhinorrhea), skin symptoms (eczema, urticaria), gastrointestinal symptoms (diarrhea, vomiting, bloody stool), and anaphylaxis. Using the sera of the patients, total serum IgE was measured by enzyme linked immunoassay (total IgE ELISA kit, Genesis, Germany). Also, immunoblot (RIDA Allergy screen test, R-Biopharm, Germany) was used for determining specific IgE against total cow's milk proteins (α -lactalbumin, β -lactoglobulin, casein and bovine serum albumin). A 0-6 grade was considered according to RIDA Allergy screen kit for specific IgE concentrations, and the amount of IgE more than 0.35 IU/ml was classified as positive in our analyses.

Statistical Analysis

The concentrations of total and specific IgEs to different cow's milk proteins were reported through median with the 1st and 3rd quartiles (Q1, Q3). The frequency of the symptoms in the study subjects and positive specific IgE tests to different CMPs were recorded by count and percentage. The relationship between the different symptoms and specific IgE test results (positive/negative) to different CMPs were assessed using Pearson's chi-squared or Fisher's exact test as appropriate. *p*-values less than 0.05 were considered statistically significant and analyses were performed using SPSS software version 18.

RESULTS

Among 87 patients, 53 (60.9%) were male and the median age was 2.5 yrs (Min: 3m- Max: 18y). Most of the participants were under 3 years of age. The median concentration of total IgE was 83.60 IU/ml [(Q1, Q3)= (13.35, 398.15)]. The median concentrations of serum specific IgEs to CMPs have been shown in table 1. The frequencies of different symptoms in patients were as

The Most Common Cow's Milk Allergenic Proteins

follows: respiratory symptoms (n=50, 63.3 %), skin symptoms (n=44, 55.7%), gastrointestinal symptoms (n=16, 20.3%), and anaphylaxis (n=11, 13.4%). Positive specific IgE to total cow's milk, α -lactalbumin, casein, β -lactoglobulin, and bovine serum albumin were detected in 75(89.3%), 65(77.4%), 64(75.3%), 52(62.7) %, and 35(44.9%), respectively. Positive specific IgE to different symptoms is shown in table 2. Accordingly, α -lactalbumin and casein were the most common allergenic proteins in the patients. There were a significant relationships between positive specific IgE to β -lactalbumin and anaphylaxis ($p=0.04$) as well as between positive specific IgE to casein and respiratory ($p=0.01$) and skin ($p=0.003$) symptoms. There were positive specific IgEs to β -lactoglobulin in 90% of the patients with anaphylaxis (Table 2).

DISCUSSION

In this study, the most common allergenic proteins of cow's milk in patients referred to IAARI with CMA were found as α -lactalbumin and casein. There were significant associations between β -lactoglobulin with anaphylactic reactions and also casein with respiratory reactions and skin reactions.

In a study by Pourpak et al, 44.5% of the children with atopic dermatitis were sensitive to cow's milk allergens.¹² The strong positive correlation between

CMA and high levels of specific IgE to CMP has been shown although it can also be detected in non allergic patients.¹³

Evidences have shown that CMA symptoms usually disappear during the first three years of life in most patients.¹⁴ We found that the frequency of CMA in less than 3 years was higher than those the other age groups which is compatible with the results of a study by Matsumoto et al.¹⁵

In parallel to our study, Ngamphaiboon et al reported that the respiratory symptoms were the most common manifestations of CMA in allergic patients. The prevalence of anaphylactic reactions in their study¹⁶ was lower than our report (0.2 versus 13.4%), which may be due to the high rates of anaphylactic patients referring to IAARI. Cow's milk has been known as the most common reason of food anaphylaxis in Iranian children, thus it is necessary to find out the causal factors of this fatal reaction.¹⁷

Patients with CMA may suffer from a range of clinical symptoms from mild cutaneous signs to fatal reactions such as anaphylaxis.^{6,7} Santos, et al. found cutaneous (81%), gastrointestinal (55%), respiratory (16%) and anaphylactic symptoms (3%) in their patients that is inconsistent with our study,^{18,24} while Restani, et al. showed that the prevalence of anaphylaxis, asthma, and gastrointestinal manifestations was 12.8%, 20.8%.¹⁹

Table 1. Concentrations of serum specific IgE to different cow's milk proteins in patients with cow's milk allergy (CMA)

Proteins	Concentration of serum specific IgE (IU/ml) Median (Q1, Q3)
Whole Cow's milk	1.36 (0.6, 3.66)
α -lactalbumin	1.36 (0.45, 11.11)
β -lactoglobulin	1.35 (0.0, 8.34)
Caseins	1.12 (0.33, 3.09)
Bovine Serum Albumin	0.21 (0, 2.62)

Table 2. Clinical symptoms and different cow's milk proteins in patients

Symptoms	Proteins n (%), p -value				
	Whole cow's milk	α -lactalbumin	β -lactoglobulin	Casein	Bovine serum albumin
Respiratory	43(91.5), 0.2	37(78.7), 0.6	27(58.7), 0.3	40(83.3), 0.01*	19(42.2), 0.5
Skin	36(83.7), 0.1	35(79.5), 0.8	31(72.1), 0.05	27(61.4), 0.003*	19(48.7), 0.4
Gastrointestinal	15(100), 0.1	11(68.8), 0.1	10(66.7), 0.7	13(81.2), 0.4	7(53.8), 0.4
Anaphylaxis	9 (90), 0.8	9(81.8), 0.7	9(90), 0.04*	8 (72.7), 0.9	6(60), 0.2

* Statistically significant

According to our knowledge, till now, there is no report about the relationship between specific IgE to cow's milk proteins and different symptoms in Iran. In a study by Shek² caseins were either the most allergenic IgE-binding or antigenic IgA, IgG –binding protein in patients with CMA. In contrast to the study by Shek, α -lactalbumin was the most allergenic protein in our study; however, we found no significant relationship between α -lactalbumin and different allergic symptoms. In a study by Natale, et al. the prevalence of patients sensitive to β lactoglobulin and BSA was 45% without any response to α -lactalbumin⁷ while in our study, 77.4% of the subjects had positive specific IgE to α -lactalbumin. Similar to the present study, Fu-Mei Chen reported α -lactalbumin as the most common allergenic protein of cow's milk in children in Taiwan.²⁰ The role of α -lactalbumin in CMA is unclear and review of the literature shows a prevalence of 0 to 80% for patients with CMA sensitized with this protein. These contradictory findings could be because of using different methods for detection of the sensitization.¹⁰ It seems that the percentage of sensitivity to β -lactoglobulin, α -lactalbumin and total casein reported by Restani et al are consistent with our findings.¹⁹

Four type of caseins including α , β , γ and κ constitute 80% of cow's milk proteins.²⁰ Different studies showed casein as the most common allergenic protein in Cow's milk.^{19,21} In our study, casein was the second common allergen in cow's milk and there was a significant relationship between specific IgE to casein with respiratory and skin symptoms.

The lack of β -lactoglobulin in human milk and its high stability makes it as the major whey allergen in cow's milk.²² In previous studies, the prevalence of patients sensitive to this protein was 13 to 76%¹⁰ which is consistent with our findings (63.2%).

Although caseins and β -lactoglobulin are the main cow's milk allergens, other minor proteins such as immunoglobulins and BSA can develop allergic reactions, too.¹⁹ Since there are major allergens in milk, and the presence of serum albumin in all mammals, the evaluation of clinical relationship to the minor allergen such as BSA is difficult in patients with CMA.²³ Moreover, asthma and rhinoconjunctivitis are detected in persistent sensitized patients to BSA.²⁴ In our study, we found no significant relationship between specific IgE to BSA and different symptoms.

The limitation of our study is that the RIDA Allergy Screen test is a semi-quantitative test, although this test has good sensitivity and specificity values. On the other hand, our cases were the patients who referred to IAARI and they were not selected from the CMA patients in Iran.

Since the sensitization to β -lactoglobulin has significant relationship to anaphylaxis, using formula free of β -lactoglobulin is suggested for children with CMA.

According to our findings, although α -lactalbumin and casein are the most common allergenic proteins of cow's milk, more anaphylactic reactions are observed in patients with a positive test for specific IgE to β -lactoglobulin. Therefore more precautions should be considered about the anaphylactic reactions in patients with positive β -lactoglobulin specific IgE test.

ACKNOWLEDGEMENTS

The authors wish to extend special thanks to Dr. Mahshid Sirjani, Mrs. Parisa Dashti and Dr. Laleh Nikfarjam for their outstanding collaborations.

REFERENCES

1. 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.
2. Shek LP, Bardina L, Castro R, Sampson HA, Beyer K. Humoral and cellular responses to cow milk proteins in patients with milk-induced IgE-mediated and non-IgE-mediated disorders. *Allergy* 2005; 60(7):912-9.
3. Barzegar S, Rosita A, Pourpak Z, Bemanian MH, Shokouhi R, Mansouri M, et al. Common causes of anaphylaxis in children: the first report of anaphylaxis registry in Iran. *World Allergy Organ J* 2010; 3(1):9.
4. Savilahti EM, Saarinen KM, Savilahti E. Specific antibodies to cow's milk proteins in infants: effect of early feeding and diagnosis of cow's milk allergy. *Eur J Nutr* 2010; 49(8):501-4.
5. Teymourpour P PZ, Fazlollahi MR, Barzegar S, Shokouhi R, Akramian R, Movahedi M, Mansouri M, Mirsaedghazi B, and Moin M. . Cow's Milk Anaphylaxis in Children First Report of Iranian Food Allergy Registry. *Iran J Allergy Asthma Immunol* 2012; 11(1).
6. Gaudin JC, Rabesona H, Choiset Y, Yeretssian G, Chobert JM, Sakanyan V, et al. Assessment of the

The Most Common Cow's Milk Allergenic Proteins

- immunoglobulin E-mediated immune response to milk-specific proteins in allergic patients using microarrays. *Clin Exp Allergy* 2008; 38(4):686-93.
7. Natale M, Bisson C, Monti G, Peltran A, Garoffo LP, Valentini S, et al. Cow's milk allergens identification by two-dimensional immunoblotting and mass spectrometry. *Mol Nutr Food Res* 2004; 48(5):363-9.
 8. Avila Castanon L, Hidalgo Castro EM, del Rio Navarro BE, Sienra Monge JJ. [Cow-milk's protein allergy]. *Rev Alerg Mex* 2005; 52(5):206-12.
 9. Wal JM. Cow's milk proteins/allergens. *Ann Allergy Asthma Immunol* 2002; 89(6 Suppl 1):3-10.
 10. Fiocchi A, Brozek J, Schünemann H, Bahna SL, von Berg A, Beyer K, et al. World Allergy Organization (WAO) Diagnosis and Rationale for Action against Cow's Milk Allergy (DRACMA) Guidelines. *World Allergy Organ J* 2010; 3(4):57.
 11. Martorell A, Garcia Ara MC, Plaza AM, Bone J, Nevot S, Echeverria L, et al. The predictive value of specific immunoglobulin E levels in serum for the outcome of the development of tolerance in cow's milk allergy. *Allergol Immunopathol (Madr)* 2008; 36(6):325-30.
 12. Pourpak Z FA, Mahmoudi M, Movahedi M, Ghargozlou M, Kazemnejad A, Eslamnoor B. The Role of Cow Milk Allergy in Increasing the Severity of Atopic Dermatitis. *Immunological Investigations* 2004; 33(1):69-79.
 13. Kneepkens CM, Meijer Y. Clinical practice. Diagnosis and treatment of cow's milk allergy. *Eur J Pediatr* 2009; 168(8):891-6.
 14. Sletten GB, Halvorsen R, Egaas E, Halstensen TS. Changes in humoral responses to beta-lactoglobulin in tolerant patients suggest a particular role for IgG4 in delayed, non-IgE-mediated cow's milk allergy. *Pediatr Allergy Immunol* 2006; 17(6):435-43.
 15. Matsumoto N, Okochi M, Matsushima M, Kato R, Takase T, Yoshida Y, et al. Peptide array-based analysis of the specific IgE and IgG4 in cow's milk allergens and its use in allergy evaluation. *Peptides* 2009; 30(10):1840-7.
 16. Ngamphaiboon J, Chatchatee P, Thongkaew T. Cow's milk allergy in Thai children. *Asian Pac J Allergy Immunol* 2008; 26(4):199-204.
 17. Teymourpour P, Shokouhi R, Fazlollahi MR, Movahedi M, Mansouri M, Mirsaeedghazi B, et al. Factors Affecting the Severity of Cow's Milk Anaphylaxis. *Iran J Allergy Asthma Immunol* 2013; 12(2):190-1.
 18. Santos A, Dias A, Pinheiro JA. Predictive factors for the persistence of cow's milk allergy. *Pediatr Allergy Immunol* 2010; 21(8):1127-34.
 19. Restani P, Ballabio C, Di Lorenzo C, Tripodi S, Fiocchi A. Molecular aspects of milk allergens and their role in clinical events. *Anal Bioanal Chem* 2009; 395(1):47-56.
 20. Chen F-M, Lee J-H, Yang Y-H, Lin Y-T, Wang L-C, Yu H-H, et al. Analysis of α -lactalbumin-, β -lactoglobulin-, and casein-specific IgE among children with atopic diseases in a tertiary medical center in northern Taiwan. *J Microbiol Immunol Infect* 2014; 47(2):130-6.
 21. Nakano T, Shimojo N, Morita Y, Arima T, Tomiita M, Kohno Y. Sensitization to casein and beta-lactoglobulin (BLG) in children with cow's milk allergy (CMA). *Arerugi* 2010; 59(2):117-22.
 22. Hochwallner H, Schulmeister U, Swoboda I, Spitzauer S, Valenta R. Cow's milk allergy: From allergens to new forms of diagnosis, therapy and prevention. *Methods* 2014; 66(1):22-33.
 23. Liccardi G, Asero R, D'Amato M, D'Amato G. Role of sensitization to mammalian serum albumin in allergic disease. *Curr Allergy Asthma Rep* 2011; 11(5):421-6.
 24. Chruszcz M, Mikolajczak K, Mank N, Majorek KA, Porebski PJ, Minor W. Serum albumins—Unusual allergens. *Biochimica et Biophysica Acta (BBA)-General Subjects* 2013; 1830(12):5375-81.