



Comparison of Cross Reactions Between Cow's Milk and Other Mammals' Milk Using Skin Prick Test and Atopy Patch Test in Children with Atopic Dermatitis and Cow's Milk Allergy

Özlem SANCAKLI¹, Ayşe YENİGÜN², Tuba TUNCEL³

¹ Department of Pediatric Allergy Immunology, Başkent University, Zübeyde Hanım Research Hospital, Izmir, Turkey

² Department of Pediatric Allergy, Izmir Kent Hospital, Izmir, Turkey

³ Department of Children's Health and Diseases, Izmir Katip Celebi University, Tepecik Education and Training Hospital, Izmir, Turkey

Corresponding Author: Özlem SANCAKLI ✉ sancakliozlem@yahoo.com

ABSTRACT

Objective: The use of other mammals' milk as an alternative treatment of cow's milk allergy is controversial due to their similar protein structures. In the present study, we aimed to investigate cross reactions with sheep's, goat, and camel's milks using skin prick test and atopy patch test in children with cow's milk allergy.

Materials and Methods: Our study group was composed of patients with atopic dermatitis who were diagnosed with cow's milk allergy in our former study where we investigated the sensitivity of different diagnostic tests commonly used to determine cow's milk allergy. In all patients, cow's milk, sheep's milk, goat's milk and camel's milk were used for skin prick test and atopy patch test. The study data were analyzed with SPSS 20.0 for Windows.

Results: Among the patients with cow's milk allergy, 15 (63%) had early-onset and 9 (37%) late-onset reactions with provocation tests. Seven (70%) of 10 children who were found skin prick test positive with cow's milk were shown to have a cross reaction against goat's and sheep's milk with skin prick test; 8 (88.8%) of 9 children who were found to be atopy patch test positive with cow's milk had a cross reaction against goat's milk, and 7 (66.6%) against sheep's milk. No patient with cow's milk allergy had a cross reaction with camel's milk with either skin prick test or atopy patch test.

Conclusion: Our study demonstrated that a cross reaction occurs at a quite high rate between cow's milk, sheep's milk and goat's milk as revealed by both skin prick testing and atopy patch test, whereas there was no demonstrable cross reaction between cow's milk and camel's milk by either method.

Keywords: Atopic dermatitis, cow's milk allergy, children, cross reactions

INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory skin disorder. The co-occurrence of AD and food allergy is particularly common among infants (1). The most commonly encountered food allergens are milk and egg (2). Food allergies are divided into two groups by their mechanism of development as early onset-type and late onset-type reactions (3). In AD, allergic

reactions are classified as mixed type, which can develop via immunoglobulin E (IgE) and or non-IgE mediated mechanisms. A single diagnostic method may not be adequate to determine the etiology since IgE and/or non-IgE mediated mechanisms are effective together in the pathophysiology of atopic dermatitis. In early-onset type reactions, skin prick test (SPT) and specific IgE (sIgE) algorithms are well established; however, there are still unclarified areas in the diagnosis of late-type reactions (3).

Atopy patch test (APT) can be used in the diagnosis of late-type reactive food allergies (4,5). The gold standard testing method for food allergies formed by both types of reaction is the double-blind placebo-controlled food provocation (DBPCFP) test (6).

Total elimination of cow's milk protein from a child's diet and providing a suitable, nutritional, substitute supply for feeding are the only current strategies (3). In children with cow's milk allergy (CMA), amino acid-based formulas or fully hydrolyzed formulas are usually used as part of an elimination diet. Although their nutritional value is high, their high cost and poor palatability for some children limit the use of extensively hydrolyzed formulas. For these reasons, there has been continuous search for other non-bovine, mammalian milk as replacement for cow's milk. Unfortunately, it has been demonstrated, by several studies, that children with CMA develop allergy to the milk proteins of these mammalian milk due to some similarity between the proteins of these mammalian milk and that of the cow's milk (7,8). However, those studies have usually been performed in children with CMA and early onset type reactions. It is unclear whether similar cross reactions occur in mixed or late onset type reactions.

In the present study we aimed to investigate, by using SPT and APT, whether cross reactions with goat's, sheep's, and camel's milks existed at similar rates in early- and late-onset reactions among children with AD and CMA.

MATERIALS and METHODS

Our study group was composed of children younger than 2 years of age with atopic dermatitis, who were recruited for an earlier study conducted by our team, where we studied sensitivity of diagnostic tests for CMA (9). In the first study atopic dermatitis diagnosis was made by using Hanifin-Rajka criteria (10). All families were informed about the study and their informed consent was obtained. Patients with reported AD flare-ups and/or allergic reactions following the consumption of cow's milk and milk products were included in the study. The patients with previously diagnosed or suspected food allergies other than cow's milk were excluded. Demographic properties, complaints, family and personal history were recorded. Having at least one family member diagnosed with conditions like as asthma, allergic rhinitis and/or conjunctivitis, AD, food and drug allergies was considered as a positive family history for atopy. Nutritional history

of the patients included information about the duration of breast-feeding and the beginning of cow's milk and milk products consumption.

Skin Prick Test (SPT)

In AD patients with active skin reactions suitable treatment modalities were planned. In patients with receding lesions, antihistamines and systemic steroids were stopped according to suggested time periods, as were topical steroids applied to the test area. In all patients, pasteurized cow's, boiled sheep's, goat's and camel's milk were used for the prick-to-prick skin test. Histamine and saline were used as positive and negative controls, respectively. After a single drop of milk on a subject's forearm area, the epidermis was pierced using Stallerpoint® (Stallergenes, France). Reactions were reviewed after 15 minutes. The test was regarded positive after which the mean diameter of the swollen area became at least 3 mm greater than the negative control diameter (11,12).

Atopy Patch Test (APT)

Haye's Test Chamber® (0540577, Netherlands) was used for APT. Drops of pasteurized cow's, sheep's, goat's and camel's milk were administered into the test chamber and saline solution was used as negative control. The test area was then checked after 20 minutes for early reactions. The patch was worn on the application area for 48 hours. The initial review was done 20 minutes after patch removal. The patch test results were reviewed within 72 hours. Reactions were grouped according to results: -: negative; +/-: doubtful: erythema only; +, weak positive: erythema and slight infiltration; ++, strong positive: erythema, infiltration, papules; +++, very strong positive: erythema, infiltration, papules, vesicles. Irritant or doubtful reactions, including sharply demarcated confluent erythema, or reactions confined to margins without infiltration were not regarded as positive (13-16).

Open Food Challenge

Open food challenge (OFC) was performed in all patients after obtaining the informed consent forms. The patients were given a cow's milk elimination diet for two weeks before the test. Vital findings (heart rate, respiration rate, arterial blood pressure, and systemic examinations) were recorded. The necessary medications (adrenaline, antihistamines, nebulized salbutamol, inhaled and systemic corticosteroids) and equipment (oxygen mask,

nebulization and resuscitation set) were made ready at bedside for possible serious reactions that might develop during the test period. Oral provocation was performed using pasteurized cow's milk. Patients were administered 0.1 ml, 0.3 ml, 0.5 ml, 1 ml, 3 ml, 5 ml, 10 ml, 20 ml, 50 ml and 100 ml cow's milk in 15-minute periods. During the test, blood pressure, heart rate, respiration rate, itching, erythema, swelling, nausea, emesis, abdominal pain, sneezing, nose itching, nasal congestion, rhinorrhea and coughing were recorded after each dose. The test was finalized in case of any occurrence of skin symptoms (rash, urticaria, and angioedema), gastrointestinal symptoms (vomiting, proctocolitis, enterocolitis, and diarrhea) and/or respiratory symptoms (bronchospasm, dyspnea) during the test (17). Patients were monitored for 24 hours after the test at the hospital. OFC negative patients were administered 200 ml/day cow's milk for a week and were reviewed thereafter. The test was considered positive in case of severe itching and/or exacerbation of atopic dermatitis lesions in the monitoring process.

Early onset reaction was defined as a reaction that occurred within the first two hours; late onset reaction was defined as a reaction that occurred between two hours to one week. Non-reactive patients were considered as negative (3).

Our study population was composed of patients who were determined by OFC as having early and/or late reactions and diagnosed with CMA in the first study. The results of the SPT and APT tests done with cow's milk and other mammals' milk were compared with regard to cross reactions. This study has been approved by ethics committee at the Adnan Menderes University, Medical Faculty.

Statistical Analysis

The study data were analyzed with SPSS 20.0 for Windows (SPSS Inc., Chicago, Illinois) software package.

The normal distributions of quantitative variables were checked with Kolmogorov-Smirnov test. Descriptive statistics were expressed as frequency (%), mean±standard deviation, and median (minimum-maximum). The normally-distributed variables were compared between independent groups using Student's t-test whereas non-normally distributed variables were compared with Mann Whitney-U test.

RESULTS

Among 53 patients with AD and suspected CMA in our previous study, 24 patients who were detected to have CMA by OFC were enrolled. Mean age of the study group was 1.5±1.0 years and the male:female ratio was 1. Table I shows the demographic properties, dietary history, and atopy rates in the families.

Among the CMA (+) patients, 15 patients (63%) had early onset and 9 (37%) had late onset reactions. Only 66.6% of the patients diagnosed with CMA were SPT positive. None of the patients with a delayed reaction had positivity with skin testing whereas all of them had APT positivity. While none of the patients with a negative OFC had positivity with SPT, three had false positive APTs. The results of the SPT and APT tests done with other mammals' milk in CMA positive patients were shown on Table II. Only 66.7% of early-onset type OFC (+) cases were SPT positive with cow's milk, and 70% of SPT positive cases were shown to cross-react with sheep's and goat's milk. None of the cases with late-onset reactions with OFC had SPT positivity. Among patients with APT positivity with cow's milk, 88.8% had APT positivity with goat's milk and 66.6% with sheep's milk. None of the patients with CMA had SPT and APT positivity.

Although one patient was found to be CMA negative with SPT, APT and OFC, a positive reaction was observed to goat's milk with APT; and that patient was subsequently diagnosed with late type isolated goat's milk allergy.

Table I. Main demographic and clinical characteristics of the CMA positive population.

Age (mean±SD)	1.5±1.0
Male/Female (n)	12/12
Positive family history for atopic disorders n (%)	20(83,3)
Total breastfeeding duration (month) (mean±SD)	11.1±6.5
Milk and milk products initiation time (month) mean (min-max)	4(1-7)
Beginning time of symptoms of atopic dermatitis (month) mean (min-max)	4(1-7)

Table II. The results of the SPT and APT tests done with other mammals' milk in CMA positive patients.

OFC positivity	SPT positivity	APT positivity
Early positive, n=15		
Cow's milk, n (%)	10 (66.7)	3 (20)
Goat's milk, n (%)	7 (46.6)	3 (20)
Sheep's milk, n (%)	7 (46.6)	3 (20)
Camel's milk, n (%)	0	0
Late positive, n=9		
Cow's milk, n (%)	0	9 (100)
Goat's milk, n (%)	0	8 (88.8)
Sheep's milk, n (%)	0	6 (66.6)
Camel's milk, n (%)	0	0

APT: Atopy patch test, SPT: Skin prick test, OFC: Oral food challenge.

DISCUSSION

Our study results showed that a considerably high cross reaction between cow's and sheep's and goat's milk could be demonstrated with both SPT and APT, but there was no cross reaction between cow's and camel's milk demonstrable by neither SPT nor APT.

The co-occurrence of CMA and moderate-to-severe atopic dermatitis is common, particularly among young children. An elimination diet should be implemented to control the symptoms of the disease in this age group. Hydrolyzed or amino acid-based formulas are typically used instead of cow's milk. However, they are expensive; and compliance cannot be guaranteed in some children due to their unpleasant taste. Therefore, families and physicians are in active search for alternative nutritional approaches. Using other mammals' milks as an alternative to cow's milk is a hot topic of research. Cow's milk is composed of casein and whey proteins. Whey allergens are composed of alpha-lactalbumin (ALA), beta-lactoglobulin (BLG), bovine serum albumin (BSA), bovine immunoglobulins, and casein allergens (consisting of 4 different proteins: alpha_{s1}, alpha_{s2}, beta, and kappa casein). Prior studies have shown that, owing to belonging to the same family, sheep's, goat, and cow's milk have similar protein structures, and there exists a high degree of cross reactivity between them (20,21). Donkey's, horse's, pig's and camel's milk show a lesser degree of similarity since these animals belong to a different family. Many former studies have suggested that horse, donkey, and camel's milk may be used as an alternative to cow's milk in case of allergy against the latter (7,8).

The most commonly recommended milk is goat's milk in patients with CMA (18). As a result of a lesser amount of caseine and a different protein structure, it has been argued that goat's milk can be tolerated better in subjects with CMA (19). However, cross reactions between cow's and goat's milk have been shown in many studies, both *in vivo* and *in vitro* (8). Bellioni-Businco (20) reported that 92% of children with CMA could not tolerate goat's milk while Infante Pina et al. (21) reported a corresponding figure of 75%. The sensitivity of SPT may be quite lower due to an immature skin sensitivity especially in young children. In our study, only 10 of 15 patients with CMA who had an early type OFC positivity also showed SPT positivity. It has been shown that 70% of SPT positive cases with cow's milk had also SPT positivity with goat's and sheep's milk. Such higher percentages of cross reactions indicate that recommending goat's milk as an alternative to cow's milk in patients with CMA is not safe. It has even been reported that administering goat's milk may trigger anaphylaxis in some subjects with CMA (22).

Clinical signs and symptoms in atopic dermatitis may emerge by IgE and non-IgE mechanisms. Therefore, diagnosing food allergies solely with SPT without using OFC may lead to inadequate elimination and a failure to adequately control AD exacerbations due to a failure to exclude non-IgE reactions. OFC is a difficult-to-apply technique that is time-consuming for physicians and that requires a careful monitorization owing to a possibility of triggering allergic reactions. A plenty of studies has been conducted on the feasibility of the diagnostic use of APT in non-IgE reactions; however, its routine use is controversial owing to inadequate rating criteria or a

subjective assessment. All patients with delayed positivity to OFC had also APT positivity to cow's milk. However, a similar APT positivity was detected in 3 OFC early positive cases and 3 APT negative cases. APT positivity in OFC early positive cases was related to an immune mechanism being both IgE- and non-IgE-mediated in these cases. However, APT positivity in OFC negative cases was linked to an AD-related impairment of skin integrity. This suggested us that one must be careful against false positives when APT is assessed. APT positivity in all cases with delayed OFC positivity suggested that APT could be used as an ancillary diagnostic test for CMA in equivocal cases. Our study showed APT positivity with goat's milk in 8 of 9 patients with delayed OFC positivity and with sheep's milk in 6. These results suggest that cross reactivity in early reactions between other mammals' milk and cow's milk also exists for delayed reactions, and that patients that are administered other mammals' milk should be definitely followed for delayed effects.

Rare cases of isolated goat's or sheep's milk allergy have been reported in the literature. This was found to be against some proteins that are found in goat's and sheep's milk but not in cow's milk (23,24). Our study similarly demonstrated a ++++ reaction against goat's and sheep's with APT in a patient who had no CMA and who was SPT negative with goat's milk. That patient could not be applied oral provocation test with goat's milk since the patient's family gave no consent for it. Although there was no history of direct goat's milk use, the patient was found to have consumed processed meat products containing goat's milk, and the patient's signs of AD remarkably regressed after an elimination diet. It was noted that when goat's milk was reintroduced to his diet, his eczema was markedly exacerbated. These findings showed us that the patient had an isolated delayed type allergic reaction against goat's milk. A review of the literature revealed that our case was the first who had a delayed type reaction with goat's milk proved by APT.

Our study has some limitations. The results of patients detected to have a cross reaction between cow's milk and other mammal milk with APT could not be verified with OFC since parents of children with positive goat's and sheep's milk tests refused oral challenge tests knowing the possibility of high risk of allergic reaction although there is lack of evidence on the risk of reaction to goat's and sheep's milk. APT's standardization for the diagnosis

of CMA is not clear and its routine use for diagnosis is a subject of debate. However, our study group consisted of patients with mixed type allergy. In patients with delayed type allergy, test methods such as SPT and specific IgE measurement may not be adequate. Our study showed very high sensitivity of APT for delayed reactions. Our study serves as a preliminary study on this subject, and additional studies on the routine use of APT for AD are needed.

In summary, our study demonstrated that among children younger than 2 years of age who had CMA, cross reactions against goat's and sheep's milk could be shown using APT, and that none of the cases developed early or delayed type reactions against camel's milk. This study suggests that camel's milk can be regarded as a safer substitute than goat's and sheep's milk in children with CMA. In order to recommend camel's milk for children with CMA, however, many studies are needed to be conducted in the future to determine the tolerable amount of camel's milk and its nutritional adequacy among children with CMA.

REFERENCES

1. 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. *Pediatric Allergy Immunol* 2010;21(Suppl 21):1-125.
2. Eigenman PA, Sicherer SH, Borkowski TA, Cohen BA, Sampson HA. Prevalance of IgE mediated food allergy among children with atopic dermatitis. *Pediatrics* 1998;101(3):E8.
3. Sicherer SH, Sampson HA. Food allergy: Epidemiology, pathogenesis, diagnosis, and treatment. *J Allergy Clin Immunol* 2014;133(2):291-307.
4. Edwards KP, Martinez BA. Atopy patch testing for foods: A review of the literature. *Allergy Asthma Proc* 2014;35(6):435-43.
5. Mehl A, Rolinck-Werninghaus C, Staden U, Verstege A, Wahn U, Beyer K, et al. The atopy patch test in the diagnostic workup of suspected food-related symptoms in children. *J Allergy Clin Immunol* 2006;118(4):923-9.
6. Bindsvlev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J, et al. Standardization of food challenges in patients with immediate reactions to foods-position paper from European Academy of Allergology and Clinical Immunology. *Allergy* 2004;59(7):690-7.
7. Restani P, Beretta B, Fiocchi A, Ballabio C, Galli CL. Cross-reactivity between mammalian proteins. *Ann Allergy Asthma Immunol* 2002;89(Suppl 1):11-5.

8. Restani P, Gaiaschi A, Plebani A, Beretta B, Cavagni G, Fiocchi A, et al. Cross reactivity between milk proteins from different animal species. *Clin Exp Allergy* 1999;29:997-1004.
9. Sancakli O, Yenigun A, Gultekin Korkmazgil B, Tuncel T. Comparison of diagnostic tests for suspected cow's milk allergy in children with atopic dermatitis. *Minerva Pediatr* 2016; Apr 15. [Epub ahead of print]
10. Hanifin JM, Rajka G. Diagnostik features of atopic dermatitis. *Act Dermatol Venerol* 1980;60(Supp 92):44-7.
11. Lee LA, Burks W. Food allergies: Prevalence, molecular characterization, treatment/prevention strategies. *Annu Rev Nutr* 2006;26:539-65.
12. Burks W, Ballmer-Weber BK. Food allergy. *Mol Nutr Food Res* 2006;50(7):595-603.
13. Niggemann B, Reibel S, Wahn U. The atopy patch test (APT)-a useful tool for the diagnosis of food allergy in children with atopic dermatitis. *Allergy* 2000;55(3):281-5.
14. Spergel JM, Brown-Whitehorn T. The use of patch testing in the diagnosis of food allergy. *Curr Allergy Asthma Rep* 2005;5(1):86-90.
15. Turjanmaa K, Darsow U, Niggemann B, Rance F, Vanto T, Werfel T. EAACI/GA2LEN position paper: Present status of the atopy patch test. *Allergy* 2006;61(12):1377-84.
16. Heine RG, Verstege A, Mehl A, Staden U, Rolinck-Werninghaus C, Niggemann B. Proposal for a standardized interpretation of the atopy patch test in children with atopic dermatitis and suspected food allergy. *Pediatr Allergy Immunol* 2006;17(3): 213-7.
17. Niggemann B. When is an oral food challenge positive? *Allergy* 2010;65(1):2-6.
18. Tavares B, Pereira C, Rodrigues F, Loureiro G, Chieira C. Goat's milk allergy. *Allergol Immunopathol (Madr)* 2007;35:113-6.
19. Bevilacqua C, Martin P, Candalh C, Fauquant J, Piot M, Roucaayrol AM, et al. Goat's milk of defective alpha(s1)-casein genotype decreases intestinal and systemic sensitization to beta-lactoglobulin in guinea pigs. *J Dairy Res* 2001;68(2):217-27.
20. Bellioni-Businco B, Paganelli R, Lucenti P, Giampietro PG, Perborn H, Businco L. Allergenicity of goat's milk in children with cow's milk allergy. *J Allergy Clin Immunol* 1999;103: 1191-4.
21. Infante Pina D, Tormo Carnice R, Conde Zandueta M. Use of goat's milk in patients with cow's milk allergy. *An Pediatr (Barc)* 2003;59:138-42.
22. Pessler F, Nejat M. Anaphylactic reaction to goat's milk in a cow's milk-allergic infant. *Pediatr Allergy Immunol* 2004;15:183-5.
23. Ah-Leung S, Bernard H, Bidat E, Paty E, Rance F, Scheinmann P. Allergy to goat and sheep milk without allergy to cow's milk. *Allergy* 2006;61:1358-65.
24. Alvarez MJ, Lombardero M. IgE-mediated anaphylaxis to sheep's and goat's milk. *Allergy* 2002;57:1091-2.