Evaluation of some predictive parameters for baked milk tolerance in children with cow's milk allergy

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Abstract

Background: The addition of baked-milk products to the diet appears to markedly accelerate the development of tolerance to unheated-milk compared to a strict avoidance diet. Objective: The present study aims to investigate the predictors of baked milk tolerance in children with IgE-mediated cow's milk allergy. Methods: The study included 80 cases who were diagnosed with IgE-mediated cow's milk allergy upon oral food challenge (OFC) testing in our clinic. Cases who did and did not develop reactions during OFC with baked milk were compared regarding clinical and laboratory parameters. Results: The cases with cow's milk allergy were 48 male and 32 female and the average age was 7.25 ± 2.45 (3-13) months. We found that 62.5% of cases showed tolerance to baked milk in the OFC test performed with cakes containing 2.6 g milk protein. When the patients who could and could not tolerate baked milk products were compared as for test results, we detected a statistically significant difference intergroup regarding diameter of wheal in skin prick test (SPT) performed with muffin slurry, levels of sIgE for cow's milk (CM), sheep's milk (SM), goat's milk (GM), casein, and the amount of unheated milk consumed until a reaction developed in the OFC test performed with unheated milk ($p < 0.05$). Conclusion: We defined novel decision points based on for CM, SM, GM, casein sIgE levels, wheal diameter in SPT with muffin slurry, and the amount of milk ingested during OFC performed with unheated milk that may be useful in predicting outcomes to baked milk challenges.

INTRODUCTION

Food allergies are an increasingly more important public health issue in developed countries.[1,2] Today, the main treatment for individuals with a CM allergy is to eliminate milk and dairy products from their diet. However, since many foods contain milk proteins, it is difficult to prevent exposure to the milk protein by eliminating dairy products from these individuals' diet alone.[3] In addition, the strict elimination of CM from the diet affects parents' quality of life and that of their children.[4] Complete CM dietary elimination may also cause nutritional deficiencies.[5] As such, many other treatment approaches are being investigated to treat food allergies. Recently, baked dairy products have been introduced in CM allergy treatment. Studies have shown that instead of unheated CM, baked CM may be a better desensitization option due to its convenience and fewer side effects.[6-10] Regularly ingesting baked milk products may also accelerate overall CM tolerance.[6]

We aimed to determine baked milk tolerance in children with an IgE-mediated CM allergy, as well as investigated possible markers that could predict baked milk product tolerance in these children, as confirmed with an OFC test.

MATERIALS AND METHOD

Study population

Children referred with a suspicion of CM allergies to a hospital-based outpatient center were enrolled in the study. Eighty infants diagnosed with IgE-mediated allergy to CM proteins were enrolled in this prospective follow-up study. Patients were subjected to an allergological work-up consisting of a detailed history, focusing on clinical signs of food allergy, combined with the SPT, prick-to- prick test, food sIgE, allergen patch test, and OFC test. Children with chronic systemic diseases were excluded.

Oral food challenge test was performed in fifty eight patients with milk-specific IgE levels or SPT <95% predictive for clinical reactivity. In addition, these fifty-eight patients received an OFC test with SM and GM. Positive OFC test results were obtained with SM in all of 58 cases, and with GM in 46 of 58 (79.3%) cases. Forty-one of 58 cases were tolerant, but 17 cases were not tolerant to baked milk. The oral provocation test was not performed in 22 patients with milk sIgE levels or SPT > 95% predictive for clinical reactivity and a history of an allergic reaction to milk within 6 months prior.[11,12] These patients were not tested for OFC, were accepted to have CM allergy and were included in the study. In addition, oral provocation tests were not performed with SM and GM in these 22 cases. Thirteen of these 22 cases were reactive to, and 9 cases were tolerant to baked milk (Figure 1).

Skin prick test and atopy patch test

We performed in all subjects SPTs. Commercial or freshly prepared extracts were used. Commercial extracts (cow's milk) were obtained from Allerbio (Varennes en Argonne, France). The raw foods used were CM, GM, and SM. Later, SPT was performed with fresh food extracts prepared from an baked milk product. Approximately 1 gram of the muffin was thoroughly mixed with 10 ml of water.[13] In all children, atopy patch tests with native food allergens were performed.[14]

Oral food challenge

The initial dose for the OFC was 3 mg, as recommended by the European Academy of Allergy and Clinical Immunology food allergy and anaphylaxis guidelines, and a total doses were given to patients that showed no reactions to the logarithmic dose increments.[15] The maximum dose included 3 g of the protein. A total of 4.5 gr milk protein was applied. The CM challenges were conducted using cow's unheated milk.

Baked Milk Challenge Test

Parents were instructed to prepare muffins or cupcakes at home according to a specific protocol that our clinic provided. Each muffin contained 1.3 g CM protein (home-baked products containing 1 cup of milk per 1 cup of flour). The muffin was baked at 180°C for 30 minutes in an oven.[8] The OFC with baked milk was applied as recommended by the European Academy of Allergy and Clinical Immunology food allergy and anaphylaxis guidelines.[15] Muffins were administered incrementally over 75 min in six steps with 15 min in between. The dosing, as expressed as a fraction of one muffin, was 1/8, 1/8, 1/4, 1/4, 1/2 and finally 3/4, totaling 2.6 grams of milk protein. Approval for the study was obtained from our Institutional Ethics Committee, and written informed consent was obtained from the parents of each enrolled child.

Statistical Analysis

IBM SPSS version 22.0 was used for all statistical analyses. The Kolmogorov-Smirnov test was used to test the normality of variables. Comparisons of continuous variables were made with independent-samples t-test and Mann-Whitney U test as appropriate. Pearson's chi-square and linear-by-linear association tests were used with an exact test for the comparison of categorical data. Sensitivity, specificity between the classifications were determined using cut-off values calculated from the group variables and analyzed using a receiver operating characteristic (ROC) curve.

RESULTS

The patients with a CM allergy numbered 48 males and 32 females at an average age of 7.25 ± 2.45 (3–13) months. The patients with a CM allergy were further categorized into two groups based on the outcome of the baked milk challenge: those who could and those who could not tolerate baked milk. The control group consisted of 42 male and 30 female patients with a mean age of 7.55 ± 3.55 (3–24) months. There was no

significant difference between the treatment and control groups regarding age, gender, weight, and height ($p > 0.05$). The mean vitamin D levels in the treatment and control groups were 28.32 ± 13.30 ng/ml and 26.09 ± 7.46 ng/ml, respectively, with no statistically significant difference between them ($p>0.05$; Table 1). When the patients who could and could not tolerate baked milk were compared for concomitant atopic diseases and other food allergies, no statistically significant differences between groups were detected ($p > 0.05$). The other most frequently encountered food allergies were to eggs, peanuts, sesame seeds, walnuts, and wheat flour. When the patients tolerant and intolerant to baked milk were compared for symptoms developed during the oral provocation test with unheated milk, a statistically significant difference was detected only for anaphylaxis development. Similarly, a significant difference was detected between patients who could and could not tolerate baked milk in terms of living place and familial atopy history ($p<0.05$; Table 1).

We detected a statistically significant intergroup difference between the patients' who could and could not tolerate baked milk products in term of wheal diameter in the SPT performed with a muffin slurry; CM, SM, GM , and casein sIgE levels; and the amount of unheated milk consumed until a reaction developed in the OFC test ($p < 0.05$). Other parametres did not statistically significantly differ between groups ($p> 0.05$) (Table 2).

When the patients who could and could not tolerate baked milk were compared, we found that amount of unheated milk ingested until the development of reaction during the OFC test performed with unheated milk was smaller in patients not tolerant to baked milk ($p < 0001$). In addition, according to ROC curve analysis performed to analyze the tolerance to baked milk, we have shown that the cut-off value for the amount of unheated milk consumed until the development of reaction during the OFC test with unheated was [?] 458 mg with 88.2% sensitivity and 51.2% specificity. (Figure 2, Table 3). Specifically, for diameter of wheal in the SPT performed with the muffin slurry, the patients who could not tolerate baked milk had statistically significantly greater wheal diameters ($p < 0.05$). We detected a cut-off value of > 3 mm with 86.6% sensitivity and 56.0% specificity for wheal diameter in the SPT (Figure 3, Table 3). Additionally, when compared to patients tolerant to baked milk, we found higher casein sIgE levels in patients intolerant to baked milk ($p < 0.05$). We determined a cut-off value of > 3.01 for casein sIgE level, with 80.0% sensitivity and 84.0% specificity. In addition, we assessed the cut-off values of CM sIgE, SM sIgE, GM sIgE levels to predict clinical reactivity to baked milk (Table 3).

DISCUSSION

Research has shown that 65–83% of children with milk allergies can safely consume baked milk products.[8-10,17-23] Notably, Cherkaoui et al[21] detected a positive rate of 23% in patients with a CM allergy in oral provocation tests performed with baked milk. Nowak-Wegrzyn et al[8], which used muffins and waffles containing baked milk, reported an identical positive rate of 23%. In three different studies involving children with a CM allergy, an OFC test was performed with cakes containing a total of 2.6 g milk protein. In these studies, Bertnikas et al[17] at 83%, Kwan et al[24] at 60%, and Mehr et al[22] at 73% reported the indicated percentages of their participants could tolerate baked milk. Contrary to these studies, Barbosa et al[25] performed an OFC test on children with a CM allergy with cakes containing a total of 2.8 g of milk protein, reporting that 46.7% of participants tolerated baked milk. Sait et al[18] found that 34.3% of their patients tolerated baked milk in an OFC test performed with 4 cakes containing 4.5 g milk. In our study, we found that 62.5% of patients showed tolerance to baked milk in an OFC test performed with muffins containing 2.6 g milk protein.

In other extant studies, there was no significant difference between patients tolerant and intolerant to baked milk products in terms of age of symptom onset or diagnosis.[6,8,13,18,22] However, Bartnikas et al[17] reported that patients reactive to baked milk were younger age than those non-reactive to baked milk. Our study showed no significant difference between patients who did and did not tolerate baked milk in age of symptom onset or age of diagnosis. Nowak-Wegrzyn et al[8], Cherkaoui et al[21], Kwan et al[24], and Barbosa et al[25] also evaluated tolerance to baked milk products in children with a CM allergy and reported no significant difference between groups with and without a baked milk tolerance in terms of gender, comorbid atopic diseases, or familial atopy history. Similarly, our study featured no difference between the groups with and

without tolerance to baked milk in terms of gender and accompanying atopic diseases. However, there was a statistical difference between patients who did and did not tolerate baked CM in term of the place where the patients lived and the family history of atopy. All of the patients reactive to baked milk lived in the cities. We speculate that more severe CM allergies can be observed in children living in cities, based on the hygiene hypothesis.

Barbosa et al[25] showed that total IgE level, peripheral eosinophil count, CM sIgE level, and wheal diameter in an SPT performed with CM were not predictive of baked milk tolerance. Similarly, Sait et al[18] reported that CM sIgE level, wheal diameter in an SPT performed with CM, eosinophil count in peripheral blood, and total IgE level cannot predict baked milk tolerance. In contrast, Nowak-Wegrzyn et al[8] reported that the study group who tolerated baked milk had lower CM sIgE levels and a smaller SPT wheal diameter. They also found that no subjects with a milk SPT wheal diameter < 5 mm reacted to baked milk. Similarly, Bartnikas et al[17] did not detect any reaction to baked milk in patients with a wheal diameter < 7 mm in SPTs performed with CM. In our study, there was no statistically significant difference between patients who did and did not tolerate CM in terms of total IgE level, peripheral eosinophil count, or wheal diameter in an SPT with CM. In addition, contrary to other studies, we measured vitamin D, SM sIgE, and GM sIgE levels by performing prick-to-prick SPT and patch testing with CM, SM, and GM. The specific IgE CM, SM, and GM levels were higher in patients reactive to baked milk, but we did not find statistically significant differences in other parameters between patients who did and did not tolerate baked milk.

Some studies in the literature reported that patients reactive to baked CM had higher casein, a-lactalbumin, and β-lactoglobulin sIgE levels compared to those non-reactive to baked CM.[8,17-19,21]Cherkaoui et al[21] showed that casein, a-lactalbumin, and β-lactoglobulin sIgE levels were higher in children reactive to baked milk. Similarly, Nowak-Wegrzyn et al[8] found that patients reactive to baked milk had higher levels of casein and β-lactoglobulin sIgE. Caubet et al[19] also told that CM, casein, and β-lactoglobulin sIgE levels were significantly higher in patients reactive to baked milk compared to tolerant patients, writing of a cut-off value of 4.95 kUA/L with 74% sensitivity and 77% specificity for casein sIgE. In our results, we showed that patients reactive to baked milk had higher CM and casein sIgE levels compared to tolerant patients; for casein sIgE, we found that a cut-off value of 3.01 kUA/L had 80% sensitivity and 84% specificity. Related, many studies have reported that a-lactoalbumin and β-lactoglobulin sIgE levels are weak markers in predicting baked milk tolerance.[17,18,25] Barbosa et al[25]reported that low casein sIgE levels can predict tolerance in baked milk, but a-lactoalbumin sIgE and β-lactoglobulin sIgE levels cannot. Similar to the literature, we found that the a-lactoalbumin and β-lactoglobulin sIgE levels in our study were not significant predictors of baked milk tolerance.

Kwan et al[24] reported that all participants with negative SPT results performed with baked milk could tolerate OFC tests with baked milk. In this study, 91% sensitivity and 61% specificity were determined using a 4 mm cut-off value for wheal diameter produced in an SPT test performed using baked milk. In addition, Sait et al[18] found that 8 of 14 participants (57%) whose SPT results were negative tolerated baked milk. However, they reported that wheal diameter in an SPT with baked milk was a weak marker to predict baked milk reactivity, as did Mehr et al.[22] In our study, we found that wheal diameter in an SPT with a muffin slurry was greater in patients reactive to baked milk at an estimated 86.6% sensitivity and 56% specificity with a cut-off value of 3 mm.

Finally, we investigated the amount of milk protein consumed until a reaction developed in an OFC test performed with unheated milk to predict the development of tolerance to baked milk. We showed that a cut-off value of 485 mg for consumed milk featured an 88.2% sensitivity and 51.2% specificity in predicting baked milk tolerance. A recent study likewise reported that tolerance to more than 620 mg of milk protein during an OFC with unheated milk could predict baked milk tolerance at 83.3% sensitivity and 82.6% specificity.[18]

In conclusion, our study defines novel decision points based on CM, SM, GM, and casein sIgE levels; wheal diameter in an SPT with a muffin slurry; and the amount of milk ingested during an OFC performed with unheated milk. These results may prove useful in predicting the outcomes of baked milk ingestion. We also found that casein, CM, SM, and GM sIgE levels can better predict the results of an OFC test with

baked milk compared to wheal diameter in an SPT performed with CM alone. From here, future studies can validate our findings in a larger patient population.

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