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Cow's Milk Protein Allergy: Prevalence of Sensitization Using the Prick Test Method among Infants in a sub-Saharan African Country

Allergie aux protéines de lait de vache : prévalence de la sensibilisation par la méthode du prick test chez les nourrissons d'un pays d'Afrique subsaharienne

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ABSTRACT

Introduction. The first allergy to appear in infants feeding on milk that contains bovine milk protein is cow's milk protein allergy (CMPA). Its prevalence can reach up to 7.5% in some countries. In Cameroon, no study to our knowledge has been devoted to CMPA or at least sensitization to cow's milk (CM) in pediatrics. We wanted to search for sensitization to cow's milk in infants 0-24 months of age in the Yaoundé region and the factors that promote this sensitization. **Methodology.** We carried out a prospective and descriptive study in the HGOPY vaccination unit from February to April 2012 in 220 infants 0 to 24 months recruited at random. It consisted of searching for a history of atopy, signs and symptoms suggestive of allergy and carrying out prick tests with cow's milk on these infants. **Results.** Our sample included 220 infants, 51% girls and 49% boys. Infants on CM represented 72% of the study population against 28% for infants on breastmilk. The subjects less than 6 months old were the most represented (68.2%). The introduction of CM into the diet of infants was early with an average age of introduction of 13 +/- 7 days. Symptoms and signs suggestive of allergy were more frequent in the group of infants on CM (37.1%) than in the group on breastmilk (26.2%). Three infants had a positive prick test, given a sensitization rate of 1.4%. **Conclusion.** Sensitization to cow's milk as detected by the prick test is as frequent in Cameroon as in certain industrialized countries. Further studies should be carried out to find and better understand the risk factors for this sensitization.

RÉSUMÉ

Introduction. La première allergie à apparaître chez le nourrisson alimente avec du lait qui contient des protéines lactées bovines est l'allergie aux protéines du lait de vache (APLV). Sa prévalence peut atteindre 7,5 %. Au Cameroun, aucune étude à notre connaissance n'a été consacrée à l'APLV ou du moins de la sensibilisation au lait de vache en pédiatrie. Ainsi nous avons voulu rechercher la sensibilisation au lait de vache chez les nourrissons de 0-24 mois d'âge dans la région de Yaoundé et les facteurs favorisants de cette sensibilisation. **Méthodologie.** Nous avons réalisé une étude prospective et descriptive dans l'unité de vaccination de l'HGOPY de Février à Avril 2012 chez 220 nourrissons de 0 à 24 mois recrutés au hasard. Elle a consisté à rechercher les antécédents d'atopie, les signes et symptômes évocateurs d'allergie et à réaliser des prick tests au lait de vache à ces nourrissons. **Résultats.** Notre échantillon comprenait 220 nourrissons dont 51% de filles et 49% de garçons. Les nourrissons sous LV représentaient 72% de la population d'étude contre 28% pour les nourrissons sous LM. Les sujets de moins de 6 mois étaient les plus représentés (68.2%). Le taux d'AME à 1, 2, 4 et 6mois était respectivement de 62%, 51%, 38% et 28%. L'introduction du LV dans l'alimentation des nourrissons était précoce avec un âge moyen d'introduction de 13+/-7 jours. Les symptômes et signes évocateurs d'allergie étaient plus fréquents dans le groupe de nourrissons sous LV (37.1%) que dans le groupe sous LM (26.2%). Trois nourrissons avaient un prick test positif, soit un taux de sensibilisation de 1.4%. **Conclusion.** La sensibilisation au lait de vache recherchée par la méthode des prick tests est autant fréquente au Cameroun que dans certains pays industrialisés. Des études plus poussées pour rechercher les facteurs de risque de cette pathologie devraient être faite pour mieux comprendre et prendre en charge cette pathologie.

HIGHLIGHTS

What is already known on this topic

The first allergy to appear in an infant fed with milk that contains bovine milk protein is the cow's milk protein allergy (CMPA). Its prevalence is largely unknown in most Sub-Saharan Africa

What question this study addressed

The prevalence of sensitization to cow's milk in infants aged 0-24 months in the Yaounde region and the factors that promote this sensitization.

What this study adds to our knowledge

Sensitization to cow's milk as detected by the prick test (1.4%) is as frequent in Cameroon as in certain industrialized countries.

How this is relevant to practice, policy or further research.

This study provides baseline data for studies in the field of sensitization of infants to cow's milk.

INTRODUCTION

According to the World Health Organization, allergy appears amongst the 4th most common diseases in the world after cancer, cardiovascular disease and HIV / AIDS. According to some estimates, it could affect 50% of the world's population between 2035 and 2050 [1]. Several epidemiological surveys show an increase in the prevalence of allergic diseases over time [2].

Allergy, the clinical expression of sensitization, corresponds to objectively reproducible symptoms or signs, caused by exposure to a specific stimulus, at a dose tolerated by normal subjects, and initiated by an immunological mechanism. It can be respiratory, food, cutaneous, ocular or medicinal. Food allergy (FA) represents the earliest atopy amongst them. It is much more common in children than in adults with a proportion of 3 children to 1 adult [3].

Cow's milk protein allergy (CMPA) is the earliest FA in children since milk is the first food of the infant. Many studies have been devoted to childhood allergy in general and to CMPA in particular worldwide. Its prevalence is therefore estimated between 2 and 7% depending on the country [4]. According Rance in France, it occupies the 4th rank of types of FA after the egg, peanuts, fish, and represents 12.6% of them. [5]

If the main FAs are well known in industrialized countries, they have been the subject of very few studies in Sub-Saharan Africa in general and in Cameroon in particular. In Sub-Saharan Africa, Bakonde [6] in Togo (1998) studied the distribution of sensitizations in pediatric allergology consultation and found a predominance of pneumallergens and positivity in cow's milk in 3.7% of cases.

In Cameroon, to our knowledge, no study has been devoted to CMPA or even less to raising awareness of cow's milk in pediatrics. In addition, this pathology, sparingly mentioned in our context, is difficult to diagnose due to the inability to titrate and identify IgE specific to cow's milk proteins in our laboratories.

Faced with the increase in allergic diseases in the world and the westernization of our lifestyles, it therefore seemed appropriate to study the sensitization to cow's milk in infants aged 0-2 years in our environment. We therefore hypothesize that sensitization to cow's milk is rare in infants in the Yaoundé region. We searched for cow's milk sensitization in infants 0-24 months of age in the Yaoundé region.

METHODOLOGY

Population, place and duration of study

This was a prospective, descriptive cross-sectional study aimed at investigating the positivity spectrum of cow's milk skin tests in infants 0-24 months of age. The study was conducted from February to April 2012 (3 months). It took place at the vaccination unit of the gyneco-obstetrics and pediatric hospital of Yaoundé (HGOPY).

Selection criteria and sampling

Any infant taken for vaccination at HGOPY during the study period. Only infants aged 0-24 months and not taking antihistamine treatment within 7 days before inclusion were included in the study. Any infant with an infectious episode and those whose parents refused to participate in the study were excluded from the study.

Data collection procedure and tools

The children were recruited from the HGOPY vaccination unit which organizes 3 vaccination sessions per week. In this unit, the study was presented and explained to parents either collectively after the usual educational chat session, or individually. While awaiting vaccination, children whose parents agreed to participate in the study, by signing the informed consent form, benefited from cow's milk prick tests, after having completed with the or parents the questionnaire. The parents were interviewed on the age of the infants, the family and personal history, the mode of feeding practiced from birth until the moment of recruitment, the possible existence of signs and symptoms suggestive of sensitization to CM. The questions were asked in such a way as not to make the mothers feel guilty about the type of food they had chosen. The prick tests were performed on the anterior aspect of the infant's forearm or on the back in case of skin lesions on the forearm that could make it difficult to read and interpret the tests. After disinfection of the skin of the forearm or of the back with 70 ° alcohol, we applied a drop of positive control (9% codeine phosphate), a drop of negative control (glycerin solution) and a drop of fresh whole milk (unpasteurized liquid whole milk of the brand " Elle et vire " obtained in the store). All of these drops were spaced at least 3 cm apart. We then carried out a light prick through each drop deposited using a standardized single-use needle of the Stallerpoint® type, without causing bleeding. The positive control and negative control solutions used were those from the STALLERGENES® laboratory. The reading was taken 15 minutes after the pricks and the mean diameter of the papule was then measured using a transparent graduated ruler. The average diameter (Dm) was obtained by measuring the transverse diameter (D1) and the

perpendicular line to half of the transverse diameter (D2). Dm = D1 + D2 / 2. The prick test was considered positive when we had a papule with an average diameter greater than 3 mm or greater than 50% of the papule of the positive control.
An emergency kit was made available in the vaccination box, including oral and IV corticosteroids, an oral antihistamine, syringes, consumables for installation of the venous line, a pediatric saturometer, injectable epinephrine ampoules and equipment. pediatric intubation to manage possible anaphylactic reactions.

Definition of operational terms

Exclusive breastfeeding (B) : The infant received only breast milk.
Partial breastfeeding : the alternation between breastfeeding and breastmilk substitutes.
Artificial nutrition : a diet composed only of a breast milk substitute.
Cow's Milk Feeding (CM): The infant received either partial breastfeeding or exclusive artificial feeding.
Dietary diversification was defined as the introduction of non-dairy foods to an infant who was breastfed or fed with formula.
Allergic rhinitis was evoked by the association of frequent or chronic rhinitis, sneezing, and nasal pruritus.
Eczema was evoked by recurrent itchy erythematous vesicular dermatosis, in layers or in patches, involving the folds, the face or the whole body, generally associated with dry skin.
Asthma was evoked by recurrent wheezing attacks (> 2 attacks in infants) or by the sensation of chest tightness or cough on exertion, with the notion of improvement under inhaled beta 2 mimetics.

Data management and analysis

The information on the personal and family history, the clinical signs if they existed, the treatments received, the progress under this treatment as well as the results of the skin tests were recorded on a technical sheet. Statistical analysis was performed using Epi-info 3.5.3 and Excel 2007 software. Chi-square test and Fisher test were used for comparison of categorical variables and means respectively. The level of statistical significance was set at $p < 0.05$.

Ethical considerations

Parents of eligible infants were informed about the study either individually or during educational chat sessions at the immunization unit. They were free to participate or not in the study. The participation agreement was materialized by signing the informed consent form. Ethical approval was granted by the ethics committee of the Faculty of Medicine and Biomedical Sciences. Authorization to recruit was obtained from the leaders of the HGOPY. Hygiene measures were ensured by disinfecting the hands of the principal investigator, disinfecting the skin before performing the prick tests and using single-use Stallerpoint® type lancets. The benefit for the infant was to know if there is sensitization to cow's milk in him and therefore a possibility of allergy to milk. Where necessary, follow-up by the pediatric allergist at the HGOPY was proposed.

RESULTS

Characteristics of the study population

Our study population consisted of 220 infants, after excluding 5 infants, 4 of whom were taking antihistamines at the time of recruitment and 1 for not reading the tests, the mother not having waited for the time to be read. Our sample size could have been larger had it not been for the reluctance of several parents to participate in the study and the reduced number of weekly vaccination sessions at the vaccination unit (3 / week). We had almost as many girls as boys in our sample.
The infants less than 6 months accounted for more than half of our study population (68.2%) and even at this age, infants on CM were more numerous (43.5%) than infants on BM (25%). Infants on CM represented 72.2% of the study population. **(Table 1)**

Table 1 : Distribution of infants by age group, sex and type of milk consumed.

Age group	Group under CM (%)	Group under BM (%)	Total (%)
≤6 months	95 (43.2)	55 (25)	150 (68.2)
F	48 (21.8)	30 (13.6)	78 (35.5)
M	47 (21.4)	25 (11.4)	72 (32.7)
6-12 months	45 (20.5)	5 (2.3)	50 (22.8)
F	24 (11)	1 (0.5)	25 (11.4)
M	21 (9.5)	4 (1.8)	25 (11.4)
> 12 months	19 (8.5)	1 (0.5)	20 (9)
F	9 (4)	1 (0.5)	10 (4.5)
M	10 (4.5)	0 (0)	10 (4.5)
Total	**159 (72.2)**	**61 (27.8)**	**220 (100)**

F = Female M = Male CM = Cow's milk feeding BM = Exclusive breast milk

The family history of atopy in 1st degree relatives was found in 52 (23.6%) infants and 2nd degree relatives in 25 (11.4%) infants. Exclusive breastfeeding was the most frequent feeding method (72.2%) chosen by mothers at birth. The overall breastfeeding rate was 93.6% in the study population, with a mean duration of 131 +/- 93 days and a median of 45 days.
The mean age at the introduction of CM was 13 days +/- 7 days. The mean age of onset of dietary diversification was 5 months 9 days +/- 1 month 6 days. CM was introduced relatively early in infants and mothers tended to begin dietary diversification around 6 months. We had a total of 82 (37.3%) infants who had already started diversification including 8 (9.7%) before the age of 4 months, 18 (22%) between 4 and 6 months and 56 (68.3%) after 6 months. Sixty two (n=62, 28,2%) of newborns had received a feeding bottle before leaving the maternity ward, of which only 4 (6.4%) were under exclusive breast feeding.

Clinical signs

Seventy-five infants (N = 220, 34%) were symptomatic. Infants on CM were more symptomatic (n = 59, 37.1%) than infants on BM (n = 16, 26.2%) but without significant difference (p> 5%). Digestive symptoms were the most frequent in the two groups. **(Table 2)**

Table 2 Clinical signs found and Prick test result by group.

CLINICAL	Group under CM (N = 59) n (%)	Group under BM (N = 16) n (%)	*p*
DIGESTIVE	**43 (72.8)**	**11 (68.7)**	0.10
Constipation	21 (35.6)	5 (31.2)	0.50
Colic	17 (28.8)	3 (18.7)	0.30
Regurgitation	13 (22.0)	3 (18.7)	0.50
Refusal to take BM	3 (5.0)	0 (0.0)	0.40
Vomiting	1 (1.7)	0 (0.0)	0.70
Crying during feedings	1 (1.7)	0 (0.0)	0.70
Poor weight gain	1 (1.7)	0 (0.0)	0.70
CUTANEOUS	**15 (25.4)**	**4 (25.0)**	0.30
Rash	10 (17.0)	2 (12.5)	0.40
Eczema	6 (10.2)	4 (25.0)	0.40
Itching	5 (8.5)	2 (12.5)	0.10
RESPIRATORY	**13 (22.0)**	**2 (12.5)**	0.10
Chronic rhinitis	9 (15.2)	1 (6.3)	0.50
Dragging cough	3 (5.0)	1 (6.3)	0.50
Allergic rhinoconjunctivitis	1 (1.7)	0 (0.0)	0.70
PRICK TEST			
Positive	3 (1.4)	0 (0.0)	N / A

CM = Feeds including cow's milk BM = Exclusive breast milk NA = Not applicable

Determine the rate of sensitization to cow's milk in the study population

The number of positive prick tests was 3 (1.4%) in our study population with a predominance of females and infants ≤ 6 months.

No infant who had a positive prick test had a family history of atopy or passive smoking. All had been introduced to CM in the 1st month of life and infants showed mild-colic from the first days of life, not aggravated by the introduction of CM. (Table 2)

DISCUSSION

We searched for cow's milk sensitivity in a population of infants aged 0-24 months in the Yaoundé region. To do this, we recruited at random at the vaccination unit of the HGOPY, 220 infants divided into 2 groups namely that of infants exclusively fed with BM (BM) and that of infants fed with CM (CM).

Our sample consisted of 220 infants of which 51% were girls and 49% were boys. This distribution is superimposed on the distribution by sex in the population of Yaoundé as described in the 2010 population census of Cameroon [7]. Dean [8] found in his sample 52% boys and 48% girls.

The age group of less than 6 months was the most represented (68.2%). This predominance could be explained by the fact that at this age the vaccinations according to the expanded vaccination program are closer and free, while they are more spaced and paid after 6 months.

Table I also shows us that infants fed with CM represented 72% of the sample against 28% for those with BM. This predominance of infants on CM is observed even in the age group of less than 6 months (63.3% vs 36.7%) and is in contradiction with the data reported by the EDS IV [9] which showed that 63.2% of infants under 6 months were on mixed feeding (BM + AM associated with water).

A family history of atopy in1st degree relatives was found in 23.6% of infants in our study population. This proportion is far lower than those found by Dean [8] in the United Kingdom and Kjaer [10] in Denmark, who found 84% and 46.4% respectively. In view of this difference, one might think that allergic pathologies would be less frequent in our environment, as hygienist theory could explain, but also that these pathologies would be less known and less diagnosed in our context compared to industrialized countries.

The overall breastfeeding rate is 93.6% in our study population and that 72.2% of mothers had chosen exclusive breast feeding (EBF) at the maternity. This rate of EBF practice at birth contrasts with that higher in certain developed countries such as Poland (75%), the Netherlands (82%) Norway (84.6%), Germany (89.5%) Sweden (94%), Slovakia (95%) and Finland (96.3%) [11,12]. This could suggest earlier exposure of newborns to CMs in our study. This finding against EBF worsens in the 4-month-old infants in our sample, which represents only 38%, against 63.2% reported by the 2011 EDS IV [9]. This phenomenon of early exposure of infants to CM is the corollary of a less frequent practice of EBF in this age group.

Exclusive artificial feeding in our study was low (6.4%) compared to that found in France in 2003 (37.4%) [13], but it remains higher than that of the general population in Cameroon in 2011 which was 2.6% [9].

Very few breastfed infants received a breast milk substitute at maternity (6.4%) in our study, compared to 20-60% in industrialized countries [13].

Mothers tended to diversify infants' diets around 6 months, although we noted that 8 infants (9.7%) had started diversification before the age of 4 months. This rate is lower than the 52% reported by Bigot-Chantepie in France [14]. This difference could be explained, on the one hand, by the fact that feeding with breastmilk substitutes is more frequent in France than in our context, and on the other hand, by the new French recommendations which recommend a dietary diversification between 4 and 6 months whereas in Cameroon, the tendency is to encourage the start of diversification from 6 months, as recommended by the WHO.

We found that subjects in the CM group were symptomatic in 37% of cases versus 26.2% of cases for subjects in the BM group. Although this difference is not statistically significant, it could indicate that infants exposed to CM are more likely to develop adverse reactions suggestive of CMPA than those receiving BM [15].

Digestive signs and symptoms were the most frequent in the two groups, followed by cutaneous signs and respiratory signs (Table II). This strong predominance of digestive signs contrasts with the data in the literature, since several authors report a predominance of cutaneous signs in CMPA [16–19]. This could suggest the participation of possible primary lactose intolerance, the prevalence of which is estimated at 70-90% in African subjects [15] and whose clinical presentation is mainly digestive.

Studies concerning sensitization to trophallergens in a non-target population are rare in the world and almost

non-existent in our environment. We opted to use the prick test method because of its ease of execution, its reproducibility and above all its cost, compared to the determination of specific IgE which not only is an expensive method, but also not available in our context.

The sensitization rate to cow's milk was 1.4% (3 cases) in our sample. This rate is comparable to that of 0.9% observed in the analogous series by Dean [8] in the United Kingdom. It is worth noting that in a series of atopic children in Morocco, Ghadi [20] found a positivity rate on the prick test of 1% while Bakonde [6] in Togo, in a series similar to that of Ghadi finds a rate of 3.7%. In addition, Matricardi et al. [21] in Germany had found a sensitization rate to cow's milk of 3.7% using the specific IgE assay. The difference in the paraclinical methods used could explain this difference.

These data attest that the trend towards sensitization to CM observed in our series is a phenomenon that is observed in comparable proportions in Europe than in other African countries. Nevertheless, given the size of our sample, it would be desirable to confirm or refute this trend in larger series and by means of perfectly randomized case-control studies.

CONCLUSION

At the end of our study, we can say that sensitization to cow's milk sought by the prick test method is as common in Cameroon as in certain industrialized countries. Further studies to find the risk factors for this pathology should be done to better understand and manage this pathology.

Conflicts of interest

The authors declare no conflict of interest.

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Author's contributions

EEC, ANAY and ME designed the study. EEC, MR, PY wrote the protocol. EEC collected and analyzed the data. ME supervised the study at every stage. EEC and MMLE wrote the article. EEC, MMLE, EP, PY, MR, NLEE and MBG revised the article until submission for publication. All authors have given their consent to submit the article.

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