

Reported Allergy to Milk Species in Schoolchildren of Fez-Meknes Region and its Relationship to Life Environment

Fez-Meknes Bölgesi Okul Çocuklarında Süt Türlerine Bildirilen Alerji ve Bunun Yaşam Ortamı ile İlişkisi

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ABSTRACT

Objectives: This study aims to estimate the prevalence of milk allergy (MA) in a school population from Fez and its territories, and to determine its relationship to life environment.

Patients and methods: A total of 3,066 middle and high school students (1,533 boys, 1,533 girls; mean age 13.8 years; range 11-20 years) from Fez center and surrounding towns were included in the study. The subjects were performed a questionnaire with questions about personal data including gender, age, their breastfeeding status, consumption of milk and dairy products, and if there is any food causing them to experience hypersensitivity. Blood samples of 134 subjects were analyzed for immunoglobulin E-mediated reactions to raw cow's milk.

Results: The results showed that 28% of schoolchildren reported allergy to food and 6.9% to milk. Concerning animal milk species, sensibility to cow's milk was reported more (2.4%), followed by goat milk (1.4%), and camel and sheep milk (less than 1%). Milk allergy was more frequent in urban areas (6.1%) than in rural areas (5.6%). Milk allergy rate was higher in girls (8.7%) than in boys (5.1%).

Conclusion: Factors such as life style, breastfeeding status, and gender can may influence the distribution of MA in populations.

Keywords: Food allergy; life environment; milk allergy; questionnaire; schoolchildren.

ÖZ

Amaç: Bu çalışmada Fez ve civarındaki okullarda eğitim gören çocuklarda süt alerjisi (SA) prevalansı değerlendirildi ve bunun yaşam ortamı ile ilişkisi belirlendi.

Hastalar ve yöntemler: Çalışmaya Fez merkezi ve civar beldelerden toplam 3.066 ortaokul ve lise öğrencisi (1.533 erkek, 1.533 kız; ort. yaş 13.8 yıl; dağılım 11-20 yıl) dahil edildi. Deneklere cinsiyet, yaş, emzirilme durumu, süt ve süt ürünleri tüketimi gibi kişisel veriler ve aşırı duyarlılık yaşamalarına neden olan herhangi bir gıda olup olmadığı hakkında sorular içeren bir anket formu uygulandı. Yüz otuz dört denegin kan numuneleri çiğ inek sütüne karşı immünoglobülin E ile oluşan reaksiyonlar açısından incelendi.

Bulgular: Sonuçlar okul çocuklarının %28'inin gıdaya, %6.9'unun süte karşı alerji bildirdiğini gösterdi. Hayvan sütü türleri içinde inek sütüne duyarlılık daha fazla bildirildi (%2.4%; bunu keçi sütü (%1.4) ve deve ve koyun sütü (%1'den az) izledi. Süt alerjisi kentsel alanda (%6.1) kırsal alana göre (%5.6) daha yaygın idi. Süt alerjisi oranı kızlarda (%8.7) erkeklerdekinden (%5.1) daha yüksek idi.

Sonuç: Yaşam tarzı, emzirilme durumu ve cinsiyet gibi etkenler popülasyonlarda SA dağılımını etkileyebilir.

Anahtar sözcükler: Gıda alerjisi; yaşam ortamı; süt alerjisi; anket formu; okul çocukları.

Food allergies are the most commonly studied adverse food reactions because of their high frequency and the severity of their reactions even with small amounts of food. Food allergy can be mediated by immunoglobulin E (IgE) or cellular mechanisms and may present with a wide variety of symptoms in the skin, and respiratory or gastrointestinal tracts.^[1] Milk allergy (MA) is one of the main food allergies, affecting mostly infants but which may also persist through adulthood.

The reported rate of allergy to cow's milk varies, which may be attributable to different methods used for diagnosis or differences in the ages of the studied populations.^[2] In general, the frequencies of self-reported adverse reactions to cow's milk are much higher than the medically confirmed diagnoses, not only in children but also in adults.^[3]

A meta-analysis of relevant original studies since 1990 by Rona et al.^[4] showed a variation in self-reported prevalence of MA between 1.2% and 17%, whereas the prevalence in studies using a double-blind placebo controlled food challenge or an open challenge varied between 0% and 3% and in studies based on skin prick testing and IgE assessment frequencies were between 2% and 9%. Nowadays, it is reported that 0.6-2.5% of preschoolers, 0.3% of older children and teens and less than 0.5% of adults suffer from allergy to cow's milk.^[3]

The increased prevalence of MA observed during recent years was related to different factors such as breastfeeding and life environment.^[5] In this study, we aimed to estimate the prevalence of MA in a school population from Fez and its territories, and to determine its relationship to life environment.

PATIENTS AND METHODS

Population characteristics

The study was carried out between April 2014 and March 2015 at different secondary public schools and included 3,066 middle and high school students (1,533 boys, 1,533 girls; mean age 13.8 years; range 11-20 years) resident in Fez-Meknes region at the time of the study. This region includes Fez city and surrounding towns like Immouzer, Ifrane, and other small villages. The population was approached through the schools after discussions with the Fez Regional Education Authorities and Head Directors. All schools consented to participate in the study. Of the schools included in this study, 10 were high schools (eight in Fez center and two in territories of Fez) and 10 were colleges (seven in Fez center and three in territories of Fez). The reported prevalence of food allergy was established by using a questionnaire that was completed by the subjects. The study protocol was approved by the

Faculty of Medicine and Pharmacy and the University Hospital Center of Fez Ethics Committee. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Questionnaire

The subjects were surveyed by a questionnaire with questions about personal data, including gender, age, and if there is any food (fish, milk, egg, cereals, peanut, white bean, green bean, sesame, red meat, chicken, almond, chocolate, strawberry) causing them to experience hypersensitivity. Then, there were questions about their breastfeeding status, consumption of milk and dairy products, and if these cause hypersensitivity. If any current adverse reaction to cow's milk or dairy products was reported, they were asked to describe the symptoms and if they had taken any medications. We classified the reported symptoms according to affected organs after food intake as skin reactions if individuals reported rash, itching, or redness; gastrointestinal symptoms if the adverse reactions were vomiting or nausea, diarrhea, or stomach pain; and respiratory symptoms if adverse reactions were throat tightness, wheezing, coughing, shortness of breath, or nasal congestion.

Immunoglobulin E measurements

Blood samples of 134 subjects were collected and analyzed for specific IgE to raw cow's milk as described before.^[6,7] Briefly, 100 µl of the raw skimmed milk was placed in the well of enzyme-linked immunosorbent assay microplate, and then this well was saturated by borate buffered saline plus containing 2.5% of Tween 20 (BBS-Tween 20). Thereafter, 100 µL of the human serum was added and plate overnighted at 4 °C. The revelation was made by adding the anti-human IgE conjugated to peroxidase followed by incubation and addition of the o-Phenylenediamine dihydrochloride substrate. Finally, after incubation at 37 °C about 30 minutes, the absorbance was measured at 490 nm.

Statistical analysis

Chi-square test was used to compare proportions with confidence intervals set at 95%. Any value of $p < 0.05$ was considered statistically significant. All statistical analyses were performed using Excel logical.

RESULTS

A total of 3,281 questionnaires were distributed in 20 schools; 16 middle and high schools in Fez center and four in its surrounding towns. Only 3,066 of the received answers were representative of school population of Fez city and its surrounding. Of the students, 57.4% ($n=1,761$) were students in middle school and 42.6% ($n=1,306$) in

TABLE 1

Demographic characteristics of study population

Demographic variables	Total population (n=3,066)	%	Population reporting food allergy (n=860)	%	<i>p</i>
Gender					<0.00001
Male	1,533	50	361	23.5	
Female	1,533	50	499	32.5	
Education					0.02
Middle school	1,761	57.4	522	29.6	
High school	1,306	42.6	338	25.8	
Region					0.24
Urban	2,353	76.7	672	28.5	
Rural	714	23.3	188	26.3	

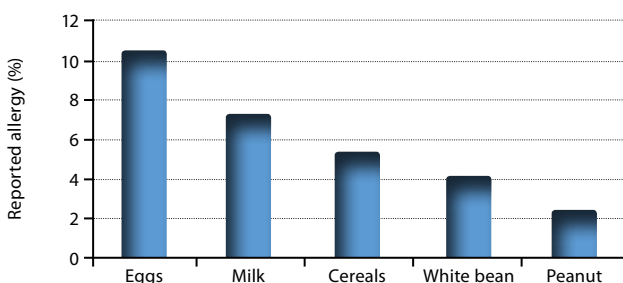
high school, 76.7% (n=2,353) were living in Fez center, while 23.3% (n=714) were living in surrounding towns.

Of the questionnaires returned, 28% (n=860) reported food allergies. According to the answers, allergy was higher in girls (n=499, 32.5%) than in boys (n=361, 23.5%). No significant differences were observed between rural and urban students, whereas, this correlation was significant regarding education level (Table 1).

The rates of reported food allergy were higher in foods of animal origin such as eggs (10.1%) and milk (6.9%) than in foods of plant origin such as cereals (5%), white bean (3.8%), and peanut (2.1%) (Figure 1). Rate of allergens of animal origin totaled to 30.7% where rate of vegetal allergens totaled to 18.6%.

Reported MA was higher in girls (8.7%) than in boys (5.1%). It was reported more by students of middle schools (8%) than students of high schools (5.5%). Depending on life environment, 6.1% reported MA in urban areas and 5.6% in rural areas (Table 2). We evaluated consumption of milk in these regions and observed that 76.6% of rural schoolchildren consume pasteurized milk compared to 84.4% in urban region.

Regarding animal milk species, cow's milk was reported more (2.4%), followed by goat's milk (1.4%) and camel's

**Figure 1.** Reported food allergy.

and sheep's milk (less than 1%) (Table 3). We measured specific IgE to different milks of different animals for 37 students. Results showed that 32.4% (n=12) of the analyzed population had more than 40 IU/mL of specific IgE to cow's milk than camel's or goat's milk (Table 4). The average of concentration of IgE to milk types was higher for cow's milk (41 IU/mL) and camel's milk (24 IU/mL) than goat's milk (17 IU/mL). No significant differences were observed for the IgE levels mentioned.

Evaluation of reported allergy to different dairy products showed that cheese, raw milk, and pasteurized milk were more common against cow's milk (3.5%, 2.6%, and 1.7%, respectively; Figure 2). It must be noted that raw milk was not consumed freshly; all subjects indicated that they boiled raw milk before consumption.

A significant finding was obtained about butter which was indicated by a great number of subjects to induce sensitivity (5.4%). The butter consumed in general was made from cow's milk and not pasteurized.

Concerning symptoms, most symptoms reported were skin reactions (4.5%), followed by respiratory

TABLE 2

Reported milk allergy according to gender, education, and region

Characteristics	Reported milk allergy	
	n	%
Milk allergy (n=3,066)	213	6.9
Gender		
Male (n=1,533)	79	5.1
Female (n=1,533)	134	8.7
Education		
Middle school (n=1,761)	141	8
High school (n=1,306)	72	5.5
Region		
Urban (n=2,353)	143	6.1
Rural (n=714)	40	5.6

TABLE 3

Reported milk allergies to different milk types (n=3,066)		
Characteristics	Reported milk allergy	
	n	%
Milk allergy	213	6.9
Milk origin		
Cow's milk	76	2.4
Goat's milk	42	1.4
Camel's milk	22	0.7
Sheep's milk	10	0.3

symptoms (1.3%), and gastrointestinal manifestations (0.9%) (Figure 3).

According to the breastfeeding status, the prevalence of self-reported food allergy was different. We observed that 24.1% of respondents who declared food allergy were naturally breastfed (BF) whereas 34.5% of respondents were not breastfed (NBF) ($p < 0.00001$). This conclusion was confirmed in reported MA with a significant correlation (Table 5).

According to the distribution of MA between different areas, MA was slightly higher in urban areas than in rural areas (6.1% vs 5.6%, respectively). Allergies to cow's and camel's milk were reported more in urban areas than in rural areas (against cow's milk: 3.6% vs 2.9%, respectively) and (against camel's milk: 0.9% vs 0.4%, respectively) (Table 6), with insignificant differences.

Anti-cow's milk IgE was found in 134 students (33 boys and 101 girls) from rural and urban areas. The results showed that the average of positive values were higher in urban areas than that in rural areas (34.7 IU/mL vs 22.2 IU/mL), and 31 urban students had higher values of more than 30 IU/mL. However, no significant differences were observed in terms of IgE levels.

DISCUSSION

Our results showed that 28% of students questioned reported that they had sensitivity to some foods. In previous reports concerning food allergy, allergy ranged from 3 to 35%,^[4] and was detected to be more common in the early years of life.^[8] On the other hand, another

research declared that the prevalence of food allergy varied from 6 to 8% in children, and it is currently increasing in many countries.^[9] A study of childhood food allergy in the United States showed that food allergy prevalence was 8%.^[10] In fact, there is a wide variation in the prevalence of food allergy worldwide, possibly due to methodological differences, demographic and cultural conditions related to food consumption habits.^[4,11] Furthermore, previous studies in western populations have established that self-reports of food allergy generally overestimate the true prevalence indicating that the true prevalence of food allergy is likely to be even lower.^[12,13] Likewise, in self-report studies, higher frequencies of food allergy are found (10%-15%) than when clinical methods such as oral provocation are used (1%-3%).^[14]

Today, milk is among the first food introduced into an infant's diet and accordingly is one of the first and most common causes of food allergy in early childhood. In our study, we found that 6.9% of the population reported allergy to milk. This result was different from that reported in USA, which showed that 21.1% of children had MA.^[10] Whereas, in France, the main food reported as causing adverse reactions was cow's milk with a rate of 11.9%.^[15] As remarked, there is a difference in reported MA between these developed countries and what we found in our country which is considered as a developing country. In fact, the prevalence of allergies is steadily increasing and seems to be associated with modern lifestyle. Therefore, it was hypothesized that high living standards and hygienic conditions are correlated with an increased risk for the development of an allergic disease.^[16-18] This is commonly referred to as the 'hygiene hypothesis' which means that the lack of intense infections in developed countries owing to improved hygiene, vaccination, and use of antibiotics may alter the human immune system such that it responds inappropriately to innocuous substances.^[19]

Depending on the sex, MA was higher in girls (8.7%) than in boys (5.1%). This is in accordance with the study of Marrugo et al.,^[20] who found that food allergy was higher in females (16.4%) than in males (12.8%). Another study conducted in USA concerning sex disparity in food allergy in 2009 showed that, among food allergic children included in the analysis, 64.3% were males and 35.6%

TABLE 4

Level of specific immunoglobulin E to milk species in human sera (n=37)

	Average of specific IgE (IU/mL)	Number of schoolchildren with IgE > 40 IU/mL	p
Anti-cow's milk	41.0	12	NS
Anti-camel's milk	23.9	9	NS
Anti-goat's milk	16.9	5	NS

IgE: Immunoglobulin E; NS: Not significant.

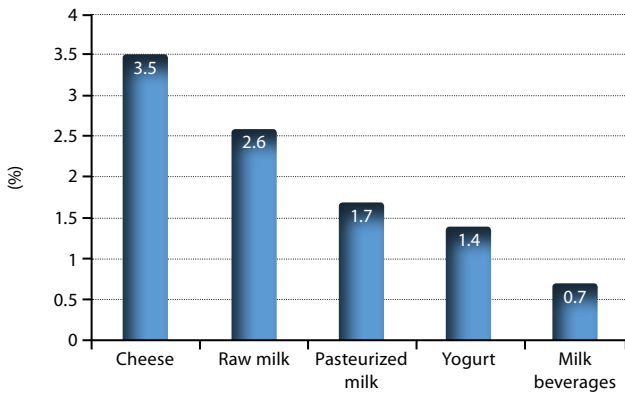


Figure 2. Reported allergies to different dairy products.

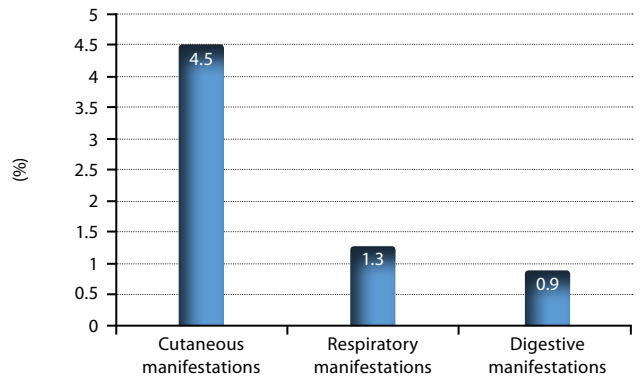


Figure 3. Reported clinical manifestations to milk allergy.

were females, whereas of the adults, 34.8% were males and 65.2% were females.^[21]

Concerning the relationship between breastfeeding and food allergy, we observed that 24.1% of respondents who declared food allergy were naturally breastfed against 34.5% of respondents who were not breastfed. The same conclusion was remarked for reported MA (BF=5.3%; NBF=9.3%). Several studies have suggested a reduced risk of allergic disease among breastfed children.^[22,23] These differences were probably related to breastfeeding duration; breastfeeding more than three months is protective and decreases risk factor for allergy.^[24] However, other investigations have not confirmed the presence of such a protective effect against allergy.^[25,26] In our study, we observed a protective effect against allergy according to our population characteristics with at least six months of breastfeeding.

Evaluation of reported allergy to different dairy products showed that raw milk and pasteurized milk were more implicated in allergy to cow’s milk (4% and

3.4%, respectively). It must be noted that raw milk was not consumed freshly but boiled before consumption.

We have observed a slight difference in prevalence of MA between rural and urban areas. It is important to remind that schoolchildren of rural towns in this study lived in conditions equivalent to schoolchildren of urban areas as indicated by consumption of pasteurized milk, and no significant differences were observed between their food habits and in their sensitization to milk. A population-based study in Mongolia demonstrated that the prevalence of allergic sensitization increased significantly with increasing urbanization: 13.6% in villages, 25.3% in rural towns, and 31.0% in the city.^[27] Likewise, several studies have shown that children grown up in rural farm environment have had a lower prevalence of allergic symptoms and atopic sensitization in childhood as compared with other rural or urban children.^[28-30] These and other data raise the possibility that allergic diseases might be related to lifestyle and degree of urbanization. However,

TABLE 5

Reported food and milk allergies according to breastfeeding status

	Breastfed population (n=1,909)		Not breastfed population (n=322)		p
	n	%	n	%	
Food allergy	461	24.1	111	34.5	<0.0001
Milk allergy	102	5.3	30	9.3	<0.01

TABLE 6

Reported milk allergy according to urban/rural areas

Origin of milk allergy	Urban (n=2,353)	%	Rural (n=714)	%	p
Cow’s milk	85	3.6	21	2.9	NS
Goat’s milk	35	1.5	16	2.2	NS
Camel’s milk	23	0.9	3	0.4	NS

NS: Not significant.

other studies failed to find significant differences between rural and urban children justifying further investigations of this phenomenon in new well-defined populations.^[31-33]

In conclusion, a high prevalence of food allergy was found in Fez center and its surrounding towns among schoolchildren. Food allergy was represented more by food from animal origin such as eggs and milk than food from plant origin such as cereals and white beans. The difference between rate of MA in urban and rural areas was not significant, whereas this result was significant between breastfed children and not breastfed children which showed the importance of breastfeeding in the avoidance of allergic diseases. Further studies are needed to investigate the relationship between MA and factors such as life style, breastfeeding status, and gender.

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