

Original article

The impact of caesarean delivery and type of feeding on cow's milk allergy in infants and subsequent development of allergic march in childhood

Background: The incidence of IgE-mediated cow's milk allergy (CMA) has increased over the last few years. There are several genetic and environmental risk factors that may be related to this allergy and the subsequent allergic march (AM).

Methods: A prospective, cohort study was conducted in patients recruited into the study between 1998 and 2002. Information on clinical variables and complementary tests, perinatal and obstetric factors and the type of hydrolysed formula used was recorded. A cross sectional study on the prevalence of allergic diseases in this cohort was performed in 2004.

Results: We compared IgE-mediated CMA patients with non-IgE-mediated CMA patients and found that IgE-mediated CMA is associated with caesarean delivery (OR = 2.14 95% CI: 1.02–4.49), duration of breast feeding (> 2 months, OR = 4.14; 95% CI: 2.17–7.89) and the use of supplementary artificial formula whilst breast feeding (OR = 2.86; 95% CI: 1.33–6.13). The factors associated with AM in IgE-mediated CMA patients were caesarean delivery (OR = 0.42; 95% CI: 0.19–0.92) and the use of more extensively hydrolysed high grade hydrolysates (+ EH/HGH) (OR = 0.44; 95% CI: 0.20–0.98), both as protective factors.

Conclusions: Caesarean delivery is demonstrated as being a risk factor for IgE-mediated CMA, but it does not increase the risk of AM in these infants. The use of + EH/HGH appears to protect IgE-mediated CMA patients from eventually developing AM.

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Allergic diseases in general are clearly on the rise in developed countries (1–3); amongst these diseases is cow's milk allergy (CMA) (4), especially that mediated by IgE. Furthermore, it has been observed that in industrialized countries non-IgE-mediated CMA (previously called cow's milk protein intolerance or cow's milk enteropathy) is decreasing rapidly, at the same time as IgE-mediated CMA is increasing (1).

Factors associated with allergies in general and to CMA in particular are diverse; amongst them are genetic factors manifested by a family history of allergy (5) or high IgE levels in the new born's umbilical cord (6). There are also many environmental factors that the new born or infant is exposed to from birth and which can affect or favour the development of an allergic response. Amongst these, the type of feeding the infant receives [breast milk vs infant artificial formula (AF)] (7, 8), exposure to infant AF in the first days of life (7, 9) and the mode of delivery [lower segment caesarean section (LSCS) vs vaginal] (10–13), have been reported.

Allergic march (AM) is defined as the natural course of atopic diseases that starts during the first months of life with food allergies and atopic dermatitis and later on in life progresses to respiratory allergies, (rhinitis and/or asthma) (14). AM starts in the first months of life with sensitization to cow's milk and egg white protein as these are the first heterologous proteins the infant is exposed to (15).

Several authors have described the influence of the intestinal flora on immune response modulation in new born infants. The initial colonization of the infant's intestine may determine subsequent immune responses, affecting both the type of response (IgE mediated) (16) and oral tolerance (17–19). Based on these findings, clinical trials have been performed on the use of probiotics in the prevention of atopic dermatitis in infants (20).

Infants born by LSCS show permanent changes in intestinal flora with less bifidogenic flora (21). Some authors found an increase in allergies in these infants (10, 11, 13, 22–26); however, there are other studies that do not support this finding (27–30).

Treatment of CMA is by elimination of the food antigen in the infant's diet and, although there may be residual antigens in the high grade protein hydrolysates (31), these formulas are the treatment of choice (32). To what extent the antigenic residual of the type of hydrolysed formula used in the treatment of IgE-mediated CMA may affect AM is not clear (33). It would appear reasonable to assume that the allergenic stimulus must be as low as possible, given that we are dealing with infants with a demonstrated capacity to produce IgE in the presence of a heterologous protein.

Objective

The aim of this study was to determine which environmental factors (perinatal and nutritional) could be associated with IgE-mediated CMA in patients with a clinical picture of CMA and which factors could predict the development of AM in children with IgE-mediated CMA.

Material and methods

A prospective, cohort study was performed in children diagnosed with CMA at the Gastroenterology and Nutrition Unit of our hospital, (maternity-paediatric referral hospital in a region of Spain with a population of approximately 600 000 inhabitants). The study was carried out between January 1998 and December 2002. Subsequently, in 2004, a study was performed to determine the prevalence of allergic diseases in this cohort of 225 patients (122 boys and 103 girls).

Study design

Cow's milk allergy was diagnosed according to the following diagnosis protocol (Fig. 1):

1. Existence of an adverse reaction after contact with cow's milk, with compatible signs and symptoms (vomiting, diarrhoea, urticaria, wheezing, angioedema, bloody stools, frequent regurgitation, rejection of food, atopic dermatitis). After improvement is demonstrated with an exclusion diet, the diagnosis is confirmed by a provocation test with AF (except in those patients with severe positive reactions and a high level of specific IgE antibodies to cow's milk proteins).
2. Determination of specific IgE antibodies to casein, alpha-lactalbumin and beta-lactoglobulin. Those patients with levels above 2.5 KU/l of one or more of the antibodies were diagnosed with IgE-mediated CMA, the rest were diagnosed with non-IgE-mediated CMA.
3. After following an exclusion diet for at least 12 months, cow's milk was re-introduced into the diet of all patients by a hospital provocation test, provided that the patients' level of specific IgE antibodies was less than 2.5 KU/l. In those patients with high levels of antibodies, the test was delayed for another 12 months when the level of specific IgE antibodies was again determined to confirm it had returned to normal.

The patients' parents were informed that this was purely an observational study and that no active interventions would be performed so that the same diagnosis/treatment protocol and clinical follow-up would be applied to all the children. None of the parents refused to allow their children to take part in the study.

At the first visit, the following variables were recorded: symptoms at diagnosis, age at diagnosis, gender, family history of atopic diseases in a first-degree relative, length of pregnancy (less or more than 37 weeks), pregnancy tolerance (poor pregnancy tolerance is defined as the occurrence of one or more of the following during pregnancy: gestational diabetes, hypertension or pre-eclampsia and threat of abortion or premature delivery), mode of delivery (caesarean section or vaginal), use of AF whilst breast feeding and length of exclusive breast feeding. The type of AF at the start of treatment, chosen by the referring paediatrician according to his/her own criteria and the tolerance to such formula from diagnosis to the first visit with the specialist, were also recorded. For practical purposes, we divided the AFs into two types: more extensively hydrolysed high grade hydrolysates (+ EH/HGH), which are those

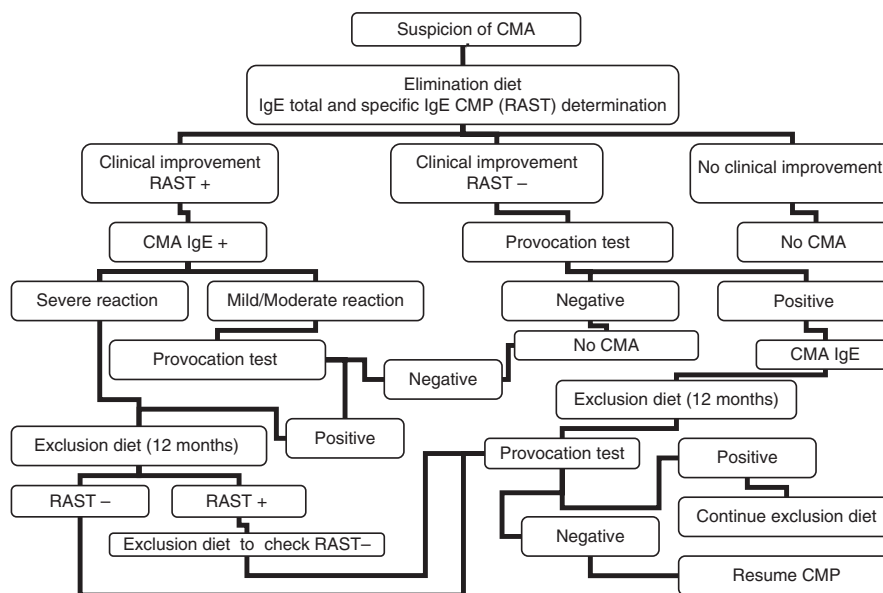


Figure 1. Cow's milk allergy diagnosis algorithm.

in which >95% of peptides are of <1000 kDa, and less extensively hydrolysed hydrolysates and soya milk formulas.

At this first visit, total IgE levels and levels of IgE antibodies specific for casein, alpha-lactalbumin and beta-lactoglobulin were determined. Subsequently, the patients were controlled according to the department's protocol until the time they were discharged (after at least one challenge test at 12 months). At these controls, information was recorded on the occurrence of allergic diseases (atopic dermatitis, other food allergies, bronchial asthma and allergic rhinoconjunctivitis).

Finally, in January 2004, a cross-sectional, systematic review of the cohort was performed by a search of the primary care and hospital computerized medical records. Information on the prevalence of allergic diseases in these patients was recorded. For practical purposes, AM was defined as the appearance or persistence over time of the following atopic manifestations: atopic dermatitis, other food allergies, asthma and allergic rhinoconjunctivitis.

Results

A total of 225 patients were included in the study of which 119 (52.9%) presented IgE-mediated CMA, whilst the rest had non-IgE-mediated CMA. Table 1 sets out the descriptive analysis of the sample, giving the means and frequencies of the variables.

On comparing the study variables according to immunological mechanism, statistically significant differences were only found as regards the mode of delivery ($P < 0.001$), use of supplementary AF whilst breast feeding ($P < 0.001$) and duration of breast feeding ($P < 0.001$). In an attempt to confirm the hypothesis that in both groups there are differences as regards certain perinatal variables, a binary logistic regression analysis was performed adjusted for the relevant study variables.

Table 1. Descriptive analysis of the cohort according to immunological mechanism

Variables*	IgE + CMA	IgE - CMA	Total
Gender (male/female), n (%)	70 (58.8)/ 49 (42.2)	52 (49.1)/ 54 (50.9)	122 (54.2)/ 103 (45.8)
Age at diagnosis (months)	4 (2.63)†	3 (4)†	4 (3.5)†
Pregnancy tolerance‡	11 (9.2)	11 (10.4)	22 (9.8)
Duration of pregnancy§	1 (0.8)	5 (4.7)	6 (2.7)
LSCS¶	67 (56.3)	30 (28.3)	97 (43.1)
AF whilst breast feeding	88 (73.9)	50 (47.2)	138 (61.3)
Duration of breast feeding (days)	105 (105)†	30 (90)†	90 (105)†
Family history of allergy	71 (59.7)	50 (47.2)	121 (53.8)
Type of AF**	53 (44.5)	43 (40.6)	96 (42.7)
Follow-up time (months)	30 (24)†	24 (25.75)†	27 (25)†

*Absolute and percentage (%) frequency values.

†Median and interquartile range (IQR) values.

‡Women with poor pregnancy tolerance (defined in *Material and methods*).

§Pregnancies of <37 weeks.

¶Caesarean delivered patients.

**More extensively hydrolysed high grade hydrolysates (defined in *Material and methods*).

Table 2. Multivariate analysis for the risk of developing IgE-mediated CMA

Variable	P (sig.)	OR	95% CI OR
Gender*	0.105	1.688	0.896 3.177
Pregnancy tolerance†	0.622	1.335	0.423 4.216
Duration of pregnancy‡	0.468	0.392	0.031 4.921
LSCS§	0.043	2.145	1.024 4.493
AF whilst breast feeding¶	0.007	2.860	1.334 6.134
Type of AF**	0.549	1.220	0.637 2.335
Duration of breast feeding††	<0.001	4.145	2.176 7.893
Family history of allergy‡‡	0.198	1.516	0.804 2.858

Categorical variables, the reference value being:

*Females compared to males.

†Good compared to poor pregnancy tolerance.

‡Full term compared to <37 weeks pregnancy.

§Vaginal compared to caesarean delivery.

¶Nonuse compared to use of AF whilst breast feeding.

**Less extensively hydrolysed high grade hydrolysates and soya milk compared to more extensively hydrolysed high grade hydrolysates.

††Exclusive breast feeding for less than 2 months compared to more prolonged breast feeding.

‡‡No family history of allergy in first-degree relative compared to family history of allergy.

The results obtained were similar to those in the univariate analysis (Table 2).

In view of these results, it is evident that the IgE-mediated CMA group and the non-IgE-mediated CMA group are heterogeneous as regards perinatal risk factors. Therefore, when determining the risk factors for developing AM, it would not seem prudent to include both disease entities in the same analysis, especially considering that from the raw analysis of AM risk, there was a much greater risk for IgE-mediated CMA patients (50/119, 42%) compared to non-IgE-mediated CMA patients (23/106, 21.7%), with an OR of 2.61 (95% CI: 1.45–4.70, $P = 0.001$). In other words, in our sample, the risk of developing AM is higher in IgE-mediated CMA patients than in non-IgE-mediated CMA patients; therefore, we decided to stratify the sample according to the immunological mechanism and study the development of AM in the two groups.

The non-IgE-mediated CMA group is a heterogeneous group whose only common characteristics are having a clinical profile compatible with CMA and having an immunological mechanism different from that mediated by IgE. In view of this, we did not consider it proper to give a prognostic opinion on AM in a group of patients in which different immunological mechanisms and therefore different risks could exist. However, their results are set out in Table 3, but we will not go into any discussion or draw any conclusions.

The IgE-mediated CMA group is, *a priori*, a homogeneous group, in which a common immunological disease mechanism exists. This enables us to draw some conclusions as regards the effect of certain perinatal and nutritional factors on the development of allergic diseases in this specific patient group. All the previously described variables were included in the statistical analysis except

Table 3. Multivariate analysis of AM risk factors in patients with non-IgE-mediated CMA

Variables	<i>P</i> (sig.)	OR	OR 95% CI	
Gender*	0.586	1.364	0.446	4.169
Pregnancy tolerance†	0.021	6.264	1.321	29.712
LSCS‡	0.648	0.706	0.158	3.155
AF whilst breast feeding§	0.685	1.328	0.337	5.228
Type of AF¶	0.301	1.906	0.562	6.460
Duration of breastfeeding**	0.755	0.822	0.239	2.818
Family history of allergy††	0.235	2.012	0.635	6.373

Categorical variables, the reference value being:

*Females compared to males.

†Good compared to poor pregnancy tolerance.

‡Vaginal compared to caesarean delivery.

§Nonuse compared to use of AF whilst breast feeding.

¶Less extensively hydrolysed high grade hydrolysates and soya milk compared to more extensively hydrolysed high grade hydrolysates.

**Exclusive breast feeding for less than 2 months compared to more prolonged breast feeding.

††No family history of allergy in first-degree relative compared to family history of allergy.

for the duration of pregnancy. The reason for this was that there were very few preterm infants in both groups and therefore it was not possible to draw any conclusions as regards this variable (Table 4).

The analytical results reveal a prominent tendency towards AM in those patients whose mothers had poor pregnancy tolerance (34) (although the differences did not reach statistical significance). Caesarean delivery and the use of +EH/HGH were shown to be protective factors for AM.

On finding that caesarean delivery was a risk factor for IgE-mediated CMA, but a protective factor for AM in this group, we decided to find an explanation for this contradiction.

Table 4. Multivariate analysis of AM risk factors in patients with IgE-mediated CMA

Variables	<i>P</i> (sig.)	OR	OR 95% CI	
Gender*	0.436	1.370	0.621	3.022
Pregnancy tolerance†	0.103	3.302	0.787	13.853
LSCS‡	0.031	0.425	0.196	0.923
AF whilst breast feeding§	0.908	0.942	0.340	2.610
Type of AF¶	0.045	0.446	0.202	0.983
Duration of breastfeeding**	0.973	1.015	0.421	2.447
Family history of allergy††	0.624	1.220	0.552	2.697

Categorical variables, the reference value being:

*Females compared to males.

†Good compared to poor pregnancy tolerance.

‡Vaginal compared to caesarean delivery.

§Nonuse compared to use of AF whilst breast feeding.

¶Less extensively hydrolysed high grade hydrolysates and soya milk compared to more extensively hydrolysed high grade hydrolysates.

**Exclusive breast feeding for less than 2 months compared to more prolonged breast feeding.

††No family history of allergy in first-degree relative compared to family history of allergy.

Table 5. Level of total IgE antibodies and specific IgE antibodies in IgE-mediated CMA patients

Antibodies	Mode of delivery	Mean	SD	<i>P</i> (sig.)
Total IgE	Vaginal	311.8000	140.24598	0.05
	LSCS	78.7800	19.50340	
Casein specific IgE	Vaginal	4.36714	1.159240	0.03
	LSCS	9.39909	2.055005	
Bet lactoglobulin specific IgE	Vaginal	3.4045	1.73373	0.08
	LSCS	7.8493	1.87485	
Alphalactalbumin specific IgE	Vaginal	1.1842	0.31549	0.01
	LSCS	3.9956	1.00923	

Significant differences between caesarean delivered infants and vaginally delivered infants in the IgE-mediated CMA group were not found for any of the variables except for the use of AF whilst breast feeding. This was much more frequent in the group of caesarean delivered infants, with an OR of 10.91 (95% CI: 3.71–32.01, $P < 0.001$). We had already seen that the percentage of infants with IgE-mediated CMA who were fed AF whilst breast feeding in our sample was very high (73.9%). However, 50% of vaginally delivered infants compared to 95% of caesarean delivered infants were fed supplementary bottles of AF.

These results led us to believe that the caesarean delivered infants were an independent subgroup within the group of patients diagnosed with IgE-mediated CMA. Therefore, we compared both the levels of total IgE at diagnosis and the levels of cow milk protein specific IgE of the two subgroups. We observed that there were significant differences, total IgE antibodies being lower and specific IgE antibodies being higher in the caesarean delivered infants (Table 5).

The other apparently protective factor (Table 4) is the use of extensively hydrolysed formulas compared to other types of formula. For practical purposes, we define +EH/HGH formulas as those in which >95% of peptides are <1000 Daltons (Nutramigen® and Pregestimil®, Mead Johnson Company, Nijmegen, Holland). The rest of the hydrolysed formulas and soya milk were included in the 'other formulas' group. The only reason to combine the soya formulas with the less extensively hydrolysed high grade hydrolysates (–EH/HGH) in the same group was not to lose sample size and statistical power in the study of other variables. When subsequently performing the same multivariate analysis comparing the +EH/HGH with the –EH/HGH, we obtained similar results with an OR of 0.376 (95% CI: 0.149–0.945, $P = 0.038$).

Discussion

The consumption of one or two bottles of AF during the first few days of life is related to the development of IgE-mediated CMA (7, 9). In our sample, 73.9% of patients had some sporadic exposure to cow's milk protein in the first few days of life. However, in the group of caesarean delivered children, 92.5% had exposure compared to 50% in the vaginally delivered children.

In the maternity department at our hospital, the feeding protocol for caesarean delivered infants during the study period was first to feed the infant AF. This was because, due to the caesarean delivery, the mother was separated from her infant during the first hours of life. Subsequently, these infants were exclusively breast fed until a mean age of 4 months at which, after this first exposure to AF, they developed CMA (35). Some authors have reasoned that it is much more harmful to expose the infant sporadically to cow's milk protein in the first few days of life than to begin with AF and continue with it, which appears to induce tolerance (36).

Studies on the correlation of mode of delivery and subsequent development of allergy are contradictory (10–13, 22–30). It has been postulated that as the caesarean delivered infant does not pass through the birth canal he or she is not inoculated with the bifidogenic microflora of the mother's vagina (37) and there would be a delay in intestinal colonization which could last throughout the first few months of life (21, 37–39). This lack of bifidogenic flora in the infant's intestine may alter the immune response in favour of Th2 lymphocytes which eventually favour the IgE response (19, 34).

Our results confirm that caesarean delivery is associated with IgE-mediated CMA. However, when we determined if patients with IgE-mediated CMA developed AM, we found that caesarean delivery was not associated with AM, but was a protective factor. In our opinion, caesarean delivery is a very important environmental factor for the sensitization to cow's milk protein together with the condition necessary to receive doses of sensitization when the new born's intestine is in sterile. Antibiotic treatment given to mothers undergoing caesarean section could be added to these factors (34). However, AM appears to be associated more with genetic factors and other environmental factors that are not present during the first moments of life.

Curiously enough, caesarean delivered CMA patients had higher levels of IgE antibodies specific for casein, beta-lactoglobulin and alpha-lactalbumin, than vaginally delivered, IgE-mediated CMA patients. Furthermore, those patients who develop AM even though they have lower levels of IgE antibodies specific for casein, beta-lactoglobulin and alpha-lactalbumin at diagnosis, have notably higher levels of total IgE antibodies. All these

data indicate that CMA in caesarean delivered patients is an allergic event that, in many cases, is isolated and for which environmental factors are much more relevant than genetic predisposition. This could explain the contradictory results of the previously mentioned authors, because if the profile of the IgE-mediated CMA patients studied is that of patients with AM, the result will be very different from those patients who do not develop AM.

Several studies have determined the protective effect that a hypoallergenic diet may have on the occurrence of allergic diseases. Although the findings are contradictory, this protective effect has been corroborated for +EH/HGH formulas and less so for –EH/HGH formulas. Studies carried out in this field are not homogeneous or comparable; the selection criteria for the study population and the definition of 'allergy prevention' are not the same, nor are the materials and methods used to activate prevention. All in all, a Cochrane review appears to bestow a more preventive potential on high grade hydrolysates although with limited evidence and does not recommend the use of partially hydrolysed casein formula or soya derived products (40, 41).

Our results demonstrated that those IgE-mediated CMA patients who were fed a +EH/HGH diet in which 95% of the peptides are <1000 Daltons (Nutramigen® and Pregestimil®), had a lower risk of developing AM compared with those patients fed –EH/HGH diets or soya milk formula. These results are consistent with the hypothesis that, in patients who have already demonstrated their capacity for an intestinal IgE response with the first heterologous food they consumed (cow's milk protein), it would be better to feed them the most hypoallergenic diet possible so as not to 'invite' these infants to continue producing IgE type responses and eventually developing AM (42).

It is obvious that the profile of the CMA patient who is likely to develop AM is very clear from the start. As regards environmental factors, it does not appear that caesarean delivery determines AM in the long run, but it does appear to be important in the initial sensitization to cow's milk protein. The type of feeding for these patients appears to be that which avoids, as far as possible, the presence of an allergen. Nutritionally, and with regard to possible AM, a diet of a +EH/HGH formula appears to be the best choice to avoid the antigenic stimulus.

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