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Role of Specific Ige Antibodies in Children with Protein Allergy

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Abstract

Allergy to cow's milk is the most common food allergy in infants and young children. Symptoms of a milk allergy reaction can range from mild, such as hives, to severe, such as anaphylaxis. The allergy is most likely to persist in children who have high levels of cow's milk antibodies in their blood. The aim of this study was to assess the value lactalbumin specific IgE and lactoglobulin specific IgE in diagnosis of cow`s milk protein allergy.

Subject and methods: This study was carried on 70 subjects classified into the following groups: Group 1**:** Include 50 patients with suspected cow milk protein allergy. Diagnosed by presence of chronic diarrhea with history of recent introduction of cow milk and positive elimination test. Group 2: Include 20 ages and sex matched apparently healthy subjects, their ages were ranged between (8-18) months. All individuals included in this study were subjected to full history taking, clinical examination , complete blood count and determination of serum total Ig E , lacto globulin and lactalbumin specific IgE which were carried out by ELISA technique.

Results: The diagnostic accuracy of lactoglobulin IgE in diagnosis of protein allergy was (84%), with sensitivity (78%), specificity (100%), positive predictive value (100%) and negative predictive value (65%) at cutoff point of 0.345 IU/ml. While, the diagnostic accuracy of lactoalbumin IgE in diagnosis of protein allergy was (83%), with sensitivity of (84%), specificity (80%), positive predictive value (91%) and negative predictive value (67%) at cutoff point of 0.335 IU/ml.

Conclusions: Lactalbumin and lactoglobulin specific IgE assay are important in diagnosis of cow milk protein allergy and their combination may give better diagnostic accuracy than total IgE assay.

Keywords: Lactalbumin; Lactoglobulin; IgE; ELISA; Immune

Introduction

Food allergy is defined as an adverse health effect arising from a specific immune response that occurs reproducibly following exposure to a given food [1].

Allergy to cow's milk protein (CMP) is an immunologically mediated reaction to one or more of the milk proteins. These proteins include caseins and whey proteins [2].

The immunological mechanisms that lead to the development of cow's milk protein allergy is not still clarified. There are different mechanisms that contribute to the pathogenesis and the main two described mechanisms at the basis of this disease refer to immediate or delayed response [3].

Cow milk allergy can be further split into IgE and non-Ig E (mostly cellular) mediated. While Ig E-mediated reactions are well recognized with validated diagnostic tests, the non IgE-mediated immune reactions are not so well defined and more difficult to recognize [4].

IgE-mediated allergy is associated with atopic manifestations such as urticaria, angioedema, vomiting, diarrhea, eczema, rhinitis, and anaphylaxis. Non-IgE-mediated allergy is associated with symptoms including gastro-esophageal reflux, vomiting, constipation, hemosiderosis, malabsorption, villous atrophy, eosinophilicproctocolitis, enterocolitis, and eosinophilic esophagitis [5].

The prevalence of food allergy (FA) varies from 6% to 8%in children, and it is currently increasing in many countries. Among all food allergens, cow's milk is one of the most common and often the first food introduced in the infant diet, even during breastfeeding. Cow's milk allergy (CMA) affects approximately 2.5% of children and may occur early in life, even during the neonatal period [6].

There is no one symptom pathognomonic of CMPA; it can present with an array of symptoms affecting different organ systems typically the skin, respiratory, and gastrointestinal tracts with many infants developing symptoms in more than one organ system [7] . There are a number of confirmatory tests which can add value when diagnosing CMPA [8].

Specific IgE testing helps to confirm diagnosis in IgE-mediated allergy, and prick tests can be used to add value to the diagnosis [5].

The aim of this study was to assess the value of serum levels of total Ig E, lactalbumin specific Ig E and lactoglobulin specific Ig E in patient suspected to have milk protein allergy.

Materials and Methods

Materials

This study was carried on 70 subjects, 50 patients with milk protein intolerance and 20 apparently health persons. They were 31 females and 39 males with age ranging from (8-18) month. The patients were attendants of out-patients clinic and inpatient of pediatrics Department,

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Menofia University Hospital during the period from April 2013 to January 2014. They were classified into the following groups:

Group 1: Include50 patients with suspected cow milk protein intolerance. They were 21 females and 29 males with mean age 11.36 ± 3.46 months. Diagnosed by presence of chronic diarrhea with history of recent introduction of cow milk and positive elimination test.

Group 2: Include 20 ages and sex matched apparently healthy subjects. They were 10 females and 10 males with mean age 10.60 ± 3.56 months.

Methods

All patients were subjected to the following:

- History taking including: History of the disease: Onset, duration, presence of prior episodes of diarrhea and its association with introduction of cow milk and history of breast feeding. Family history of milk allergy or any other type of food allergies.
- Complete clinical examination:-Measurement of the weight and height of the infant to know if the infant growth is retarded or not. Search for signs of dehydration (as sunken eyes, thirst and delay in return of abdominal skin fold) to assess the severity of diarrhea and vomiting. Examination of the skin for any urticarial rash. Auscultation of the chest wheezes to know if the respiratory tract is involved or not.
- Laboratory investigations were measured for both patients and controls including: Complete blood picture, Serum total IgE, lactalbumin specific IgE, lactoglobulin specific Ig E, total proteins and albumin levels.

Samples collection

Five milliliters (ml) of venous blood were taken from each subject and divided as follows: 2 ml were put immediately in an EDTA tube for complete blood count (CBC), while the remaining 3 ml were put in a plain tube, left to clot for 30 minutes at room temperature then subjected to centrifugation for 10 minutes at 4000 rotation per minute (rpm) and the serum obtained was put in several aliquots, stored at -8°C until the time of assay [9,10].

Assay methods

- Complete blood picture was measured with Pentra 80 automated blood counter (ABX-France-Rue du Caducee-Paris Euromedecine-BP-7290.34184 Montpellier-Cedex 4.)
- Serum total IgE was determined using solid phase enzyme-linked immunosorbent assay [9]. The kits provided by Chemux Bioscience, USA.
- Lactalbumin and lactoglobulin specific IgE was determined using a cellulose disc-based enzyme allergosorbent test (EAST) [10]. The kit provided by RIDASCREEN Germany.
- Colometeric determination of total plasma proteins [11] and albumin level [12] Diamond Egypt.

Statistical analysis

The data collected were tabulated & analyzed by SPSS (statistical package for the social science software) statistical package version 20 on IBM compatible computer. Quantitative data were expressed as mean ± standard deviation(X ± SD) and analyzed by applying T test for comparison between two groups of normally distributed variables, while for comparison between two groups of not normally distributed variables Mann-Whitney Test was applied. Qualitative data were expressed as number and percentage (No &%) and analyzed by applying chi-square test and for 2 × 2 table and one cell has expected number less than 5 fisher's exact test was applied. Spearman correlation was used for no normally distributed quantitative variables or when one of the variables is qualitative. ROC curve was used to determine cutoff points, sensitivity and specificity for quantitative variables of interest.

Results

The result of the present study is represented in tables 1-6. The results show no significant statistical difference among the two studied groups as regards age and gender distribution (not shown).

There was a significant statistical difference between the studied groups regarding family history, immediate, respiratory and skin symptoms. While non-significant difference as regarding breast feeding (Table 1). There was a significant increase of WBCs count, eosinophil% total IgE, lactalbumin specific IgE and lactoglobulinspecificIg E in group I when compared with group II. Also it shows significant decrease of hemoglobin levels and serum total proteinsin group I when compared with group II and non significant statistical difference regarding other parameters (Table 2).

The diagnostic accuracy of total Ig E in diagnosis of protein allergy was (59%), with sensitivity of (44%), specificity (95%), positive predictive value (96%) and negative predictive value (40%) at cutoff point of 50.05 IU/L. The diagnostic accuracy of lactoglobulin IgE in diagnosis of protein allergy was (84%), with sensitivity (78%), specificity (100%), positive predictive value (100%) and negative predictive value (65%) at cutoff point of 0.345 IU/ml. While, the diagnostic accuracy of lacto albumin IgE in diagnosis of protein allergy was (83%), with sensitivity of (84%), specificity (80%), positive predictive value (91%) and negative predictive value (67%) at cutoff point of 0.335 IU/ml (Table 3).

The diagnostic accuracy of combined total IgE and lacto globulin

History	Studied groups				Fisher's exact test	P value
	Cases (n=50)		Controls (n=20)			
	NO.	%	NO.	%		
Family history						
Present	20	40	0	0	9.33	<0.01
Absent	30	60	20	100		
Breast feeding						
Yes	26	52	11	55	0.05*	>0.05
No	24	48	9	45		
Immediate symptoms						
Present	24	48	0	0	12.56	< 0.001
Absent	26	52	20	100		
Respiratory symptoms						
Present	18	36	0	0	7.9	<0.01
Absent	32	64	20	100		
Skin symptoms						
Present	27	54	0	0	15.38	< 0.001
Absent	23	46	20	100		

*χ2 test

Table 1: Statistical comparison of history and clinical data suggestive of CMA among the studied groups.

Laboratory parameters	Studied groups		Test of significance	P value
	Cases (n=50)	Controls (n=20)		
Hb%(gm/dl) :	11.72 ± 1.28	13.10 ± 1.18	t= 4.13	<0.001
RBCs count(×106/L):	4.72 ± 0.34	4.88 ± 0.32	t=1.80	>0.05
Platelets count(×10³/L):	326.40 ± 84.79	344.70 ± 72.41	t=0.84	>0.05
WBCs count(×10³/L):	7.05 ± 2.37	5.74 ± 1.20	t=3.03	<0.01
Neutrophils%	62.64 ± 3.09	63.30 ± 2.07	t=0.88	>0.05
Lymphocytes%	28.06 ± 3.89	29.15 ± 2.25	t=1.17	>0.05
Monocytes%	3.44 ± 1.0751	3.05 ± 0.76	t=1.71	>0.05
Eosinophil%	4.98 ± 4.05	1.60 ± 1.05	U=3.96	<0.001
Basophils%	0.50 ± 0.51	0.65 ± 0.49	U=1.13	>0.05
Total protein(gm/dl)	7.29 ± 1.31	8.41 ± 1.88	t=2.43	<0.05
Albumin(gm/dl):	3.71 ± 0.66	3.66 ± 0.41	t=0.37	>0.05
Total IgE(IU/ml):	86.53 ± 139.66	19.07 ± 16.75	U=2.62	<0.01
LactoglobulinIgE(IU/ml):	1.04 ± 1.23	0.29 ± 0.05	U=4.91	<0.001
LactalbuminIgE(IU/ml):	0.65 ± 0.40	0.28 ± 0.06	U=5.11	<0.001

t: t test U: Mann-Whitney Test.

Table 2: Statistical comparison of laboratory parameters among studied groups.

Cutoff point	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Diagnostic accuracy
Total IgE50.05 IU/ml	44%	95%	96%	40%	59%
Lactoglobulin specific IgE 0.345 IU/ml	78%	100%	100%	65%	84%
Lactoalbumin specific IgE 0.335 IU/ml	84%	80%	91%	67%	83%

Table 3: Diagnostic validity of total IgE, lactoglobulin and lacto albumin specific IgE (IU/ml) in diagnosis of protein allergy cases.

Combinations of specific IgE antibodies	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Diagnostic accuracy
Combined total IgE and lactoglobulin	88%	50%	81%	63%	77%
Combined total IgE and lactalbumin	92%	35%	78%	64%	76%
Combined lactoglobulin and lactalbumin	90%	80%	92%	76%	87%
Combined total IgE, lactoglobulin and lactalbumin	92%	35%	78%	64%	76%

Table 4: Diagnostic validity of combinations of specific IgE antibodies in diagnosis of protein allergy cases.

was 77% with sensitivity of 88% , specificity of 50%,positive predictive value of 81% and negative predictive value 63% , the diagnostic accuracy of combined total IgE and lactalbumin was 76% with sensitivity of 92% , specificity of 35%, positive predictive value of 78% and negative predictive value64%, the diagnostic accuracy of combined lacto globulin IgE and lactalbumin was 87% with sensitivity of 90%, specificity of 80%, positive predictive value of 92% and negative predictive value76%. While, the diagnostic accuracy of combined total Ig E, lactoglobulin IgE and lactalbumin was76% with sensitivity of 92% , specificity of 35%, positive predictive value of 78% and negative predictive value 64% (Table 4).

There was a significant statistical difference between positive and negative cases of total IgE and lactoglobulin specific IgE in infant suggestive of cow milk allergy according to family history. While, There was a significant statistical difference between positive and negative cases of total IgE in infant suggestive of cow milk allergy according to immediate symptom and non-significant statistical difference according to other parameters (Table 5).

There was a significant statistical difference between positive and negative cases of total Ig E in infant suggestive of cow milk allergy according to WBCs count, eosinophil%, lacto globulin IgE and lacto albumin IgE and a significant statistical difference between positive and negative cases of lacto globulin Ig E in infant suggestive of cow milk allergy according to eosinophil% and total Ig E, RBCs count and lacto albumin IgE. Also, there was significant statistical difference between positive and negative cases of lacto albumin IgE in infant suggestive of cow milk allergy according to eosinophil%, total Ig E and lacto globulin IgE while, other parameters show a non-significant statistical difference (Table 6).

Discussion

The prevalence of CMA in children living in the developed world is approximately 2 to 3%, making it the most common cause of food allergy in the pediatric population [13].

Specific IgE testing helps to confirm diagnosis in Ig E-mediated allergy, and prick tests can be used to add value to the diagnosis. Vandenplas et al. [5] but a combination of the 2 tests is not necessary for the diagnostic workup [7].

This study asses the value of lactoglobulin and lacto albumin specific IgE in diagnosis of CMPA. In the present study neither the age nor the gender was significant [13]. As the age ranges from 6 month to18 month with mean of 11.36 month and male to female ratio was 1.3:1. This is agreeing with the studies of Castro et al. [6], Topal et al. [14], Van den Hogen et al. [15], and Robert et al. [16].

In the present study the positive family history of allergic diseases was present in 40% of cases. It is also significant in relation to lactoglobulin specific IgE and total IgE.

This agrees with the study of Sirasuda et al. [17] who found that, fifty-two percent of parents had atopic diseases. While the study of Mowszet et al. [18] stated positive family history of allergy in only 11%. A genetic basis for atopic disease is supported by twin studies which

History	Total IgE				LactoglobulinIgE				LactalbuminIgE				P value
	Positive (n=22)		Negative (n=28)		Positive (n=39)		Negative (n=11)		Positive (n=42)		Negative (n=8)		
	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	
Family history:													P1< 0.001
Present	15	68	5	18	19	49	1	9.1	19	45	1	13	P2<0.05
Absent	7	32	23	82	20	51	10	91	23	55	7	88	P3>0.05
Breast feeding :													P1>0.05
Yes	11	50	26	54	20	51	6	55	22	52	4	50	P2>0.05
No	11	50	21	46	19	49	5	46	20	48	4	50	P3>0.05
Immediate symptoms:													P1< 0.01
Present	15	68	9	32	20	51	4	36	22	52	2	25	P2>0.05
Absent	7	32	19	68	19	49	7	64	20	48	6	75	P3>0.05
Respiratory symptoms:													P1>0.05
Present	10	46	8	29	14	36	4	36	15	36	3	38	P2>0.05
Absent	12	55	20	71	25	64	7	64	27	64	5	63	P3>0.05
Skin lesions:													P1>0.05
Present	14	64	13	46	22	56	5	46	25	60	2	25	P2>0.05
Absent	8	36	15	54	17	44	6	55	17	41	6	75	P3>0.05

P1 between total IgE positive and negative cases.
P2 between lactoglobulinspecific IgE positive and negative cases.
P3 between lactoalbuminspecific IgE positive and negative cases.

Table 5: Comparison of family history and history of symptoms suggestive of CMA in infants with total IgE-positive and negative cases.

Laboratory parameters	Total IgE		LactoglobulinIgE		LactalbuminIgE		P value
	Positive (n=22) Mean ± SD	Negative (n=28) Mean ± SD	Positive (n=39) Mean ± SD	Negative (n=11) Mean ± SD	Positive (n=42) Mean ± SD	Negative (n=8) Mean ± SD	
Hb% (gm/dl)	11.47 ± 1.16	11.91 ± 1.36	11.69 ± 1.16	11.81 ± 1.70	11.56 ± 1.21	12.07 ± 1.66	P1>0.05 P2>0.05 P3>0.05
RBCs count (109 /L)	4.68 ± 0.33	4.75 ± 0.35	4.79 ± 0.35	4.52 ± 0.21	4.76 ± 0.33	4.54 ± 0.37	P1>0.05 P2<0.05 P3>0.05
WBCs count (×10³/L)	8.78 ± 2.36	5.69 ± 1.24	7.33 ± 2.48	6.06 ± 1.68	7.22 ± 2.36	6.16 ± 2.39	P1< 0.001 P2>0.05 P3>0.05
Platelets count (×10³/L)	339.18 ± 71.00	316.35 ± 94.28	324.64 ± 83.46	332.64 ± 93.29	331.69 ± 83.95	298.63 ± 89.41	P1>0.05 P2>0.05 P3>0.05
Eosinophil%	8.68 ± 3.32	2.07 ± 1.15	5.87 ± 4.16	1.81 ± 0.87	5.33 ± 3.94	3.12 ± 4.42	P1< 0.001 P2< 0.001 P3<0.05
Total protein (gm/dl)	7.65 ± 1.49	7.00 ± 1.09	7.35 ± 1.38	7.09 ± 1.03	7.35 ± 1.38	6.98 ± 0.81	P1>0.05 P2>0.05 P3>0.05
Albumin (gm/dl)	3.62 ± 0.59	3.77 ± 0.72	3.72 ± 0.67	3.66 ± 0.70	3.68 ± 0.66	3.87 ± 0.72	P1>0.05 P2>0.05 P3>0.05
LactoglobulinIgE(IU/ml)	1.56 ± 1.58	0.62 ± 0.62	----------	----------	1.15 ± 1.30	0.47 ± 0.44	P1<0.001 ------- P3<0.01
Lactalbumin IgE(IU/ml)	0.85 ± 0.44	0.49 ± 0.28	0.69 ± 0.36	0.51 ± 0.50	----------	----------	P1< 0.001 P2 <0.01 --------
Total IgE	----------	----------	106.75 ± 152.24	14.83 ± 16.07	93.93 ± 144.32	47.63 ± 111.74	-------- P2 <0.001 P3<0.01

Table 6: Comparison of laboratory investigations in infants with total IgE, lacto globulin and lacto albumin specific IgE positive and negative cases.

show that allergies such as asthma, eczema, and hay fever correlate more highly in monozygotic than dizygotic twins irrespective of whether the monozygotic twins were raised together orapart [5].

CMPA can induce a diverse range of symptoms of variable intensity in infants. It is helpful to differentiate between the "immediate" (early) reactions and "delayed" (late) reactions. Immediate reactions occur from minutes up to 2 hours after allergen ingestion and are more likely to be Ig E mediated, whereas delayed reactions manifest up to 48 hr or even 1 week following ingestion. Combinations of immediate and delayed reactions to the same allergen may occur in the same patient [19].

In the current study the presence of respiratory symptoms present in 36% of cases. The immediate symptoms and the skin symptoms were present in 48% and 54% of cases respectively. This is in accordance with the studies of Skripak et al. [20], Castro et al. [5], Sirasuda et al. [17], Robert et al. [16] and Van den Hogen et al. [15] who stated that, the most common presenting symptoms of milk allergy were skin-related reaction. In contrast to the present study Merras et al. [21] found that skin symptoms were non-significant this may be because the study focused mainly on non Ig E mediated milk allergy.

In the current study the history of breast feeding was 52% with non-significant relation to total Ig E, lactoglobulin specific Ig E and lactalbumin specific IgE. This in accordance with the study of Skripak et al. [20]. In contrast the study of Sirasuda et al. [17] found that the mean age of ceasing breast feeding only was 1.09 month (0-10), while that of starting CM formula was 1.05 month (0-10). So, exclusive breastfeeding has been shown to be the best method to prevent allergy [5].

In the current study the WBCs and Eosinophil% were significantly increased in patients group in comparing to controls. WBCs count significantly increased in cases with positive total IgE. The Eosinophil%significantly increased in cases with positive total Ig E, lactalbumin specific Ig E and lactoglobulin specific IgE. This agrees with studies of Sirasuda et al. [17] and Omeret al. [22].

In the current study the total IgE level was significantly increase in patients group in compare to controls with mean of 86.53 ± 1.23 IU/ml. The cut off value was 50.05 with sensitivity of 44% and specificity of 95%, positive predictive value of 96% and negative predictive value of 40% and diagnostic accuracy of 59%.

In contrast the study of Ahren et al. [23] stated that the mean of total IgE in baseline diagnosis was 436.9 ± 924.2 IU/ml this may be because their study population was 52 children with CMA who had at least two consecutive food challenge tests.

A positive test for specific IgE at the time of diagnosis predicts a longer period of intolerance as compared with those children who have negative tests [24].

In the present study the cutoff point of lactoglobulin specific Ig E was 0.345 IU/ml with sensitivity of 78% and specificity of 100%, the positive predictive value is 100%, the negative predictive value is 65% and the diagnostic accuracy is 84%. The cutoff point of lactalbumin specific Ig E is 0.335 IU/ml with sensitivity of 84% and specificity of 80%, the positive predictive value is 91%, the negative predictive value is 67% and the diagnostic accuracy is 83%.

This agrees with the study of Skripak et al. [20], Ahren et al. [23], Corinne et al. [25] and Sirasuda et al. [17] in which IgE-mediated disease was defined as having a skin prick test with a wheal diameter 3 mm and/or a cm (cow milk) IgE 0.35 kU/L.

In contrast with the study of Lisa et al. [26] Sensitivity, specificity, and PPV, for α-lactoalbumin and β-lactoglobulin were poor. While the NPV for β- lacto globulins IgE at 0.35 kU/L was 84.2% with AUC >90%

In contrast the study of Castro et al. [6] stated that the best specific IgE concentrations found were: 3.06 kUI/l for whole milk, 2.08 kUI/l for lactalbumin, 1.85 kUI/l for lactoglobulin and 1.47 kUI/l for casein this difference may be because this study included 123 children with confirmed CMA and the present study included only 50 children with suspected CMA.

Also, in contrast to the study of Federica et al. [27] in which the determination of cow's milk specific IgE was performed and values greater than 0.10 kUa/L were considered as positive.

In the present study the diagnostic validity of combined levels of total Ig E and specific Ig E showed that the combination of lactalbumin and lacto globulin specific IgE was the best as sensitivity was 90% , specificity was 80%, positive predictive value was 92%, negative predictive value was76% and diagnostic accuracy was 87%.

It can be concluded that lactalbumin and lactoglobulin specific IgE are important in diagnosis of cow milk protein allergy and their combination may give better diagnostic accuracy. Total IgE has lesser diagnostic use in milk protein allergy. The level of lactoglobulin and lactalbumin specific IgE are related to family history, immediate symptoms and eosinophil percentage.

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