Original article

Cow's milk protein elimination in autistic children: language, cognitive and behavioral outcome.

Background: *Behavioral modification and structured education are necessary in autism but rather insufficient. Various dietary restrictions have been suggested as important prerequisites to benefit from other interventions in this disorder.*

Objective: *This study was designed to highlight the degree of benefit in various aspects of development of autistic children upon elimination of cow's milk protein (CMP) from their diet and assess the level of specific IgE for CMP in their sera.*

Methods: *The current study was conducted on 22 autistic children who were compared to 30 age and sex matched healthy children. Enrolled autistic children were randomly assigned to one of two groups. The parents of first group were instructed to eliminate cow milk (CM) from the diet of their children throughout the study period while patients of the second group were allowed to eat without restrictions. Each enrolled child was subjected to complete dietetic history taking, clinical examination and measurement of IgE for CM antigen in their sera by enzyme immunoassay. Autistic patients underwent a Childhood Autism Rating Scale (CARS) test. The patients were also subjected to language and intelligent quotient (IQ) testing, social and mental age assessment and child psychiatric evaluation. The autistic children received an interventional program for six months and were then re-evaluated using the previous clinical parameters.*

Results: *The first group achieved significantly lower CARS test results ($p<0.01$), significantly higher language age ($p<0.05$) and significantly higher rate of change of CARS, language, social age, mental age and IQ ($p<0.001$, <0.05, <0.05, <0.01 and <0.05 respectively) compared to the second group after 6 months of follow up. There was also a significantly higher mean specific IgE level to CMP in the autistic patients as compared to the controls. Additionally, 45.5% of patients who were on CM elimination diet went one CARS category down compared to only 36.4% of the second group.*

Conclusion: *We report improvement in language, cognition and behavioral capabilities upon CM elimination in a group of autistic children. The higher CM specific IgE in these children may suggest that such adverse reaction to CM may have an allergic basis. Wider scale studies are needed to justify this adjuvant therapeutic option in autistic children hoping for better achievement from the current interventional programs.*

Key words: Allergy – Autism – CARS – Cow milk – IgE – IQ.

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INTRODUCTION

Autism, or rather autism spectrum disorders (ASD), is a childhood psychiatric problem characterized by a qualitative impairment in verbal and non-verbal communication, in imaginative activity, and in reciprocal interactions[1]. It is the most prevalent of a subset of disorders organized under the umbrella of pervasive developmental disorder (PDD)[2]. This neurodevelopmental disorder has many biological causes, including genetic, syndromic and environmental[3].

Some authors have previously suggested that food peptides might be able to determine toxic effects at the level of the central nervous system (CNS) by interacting with neurotransmitters. In fact, a worsening of neurological symptoms has been reported in autistic patients after the consumption of milk and wheat[4]. The suggested toxins are the Casomorphins and Gliadomorphins

which are families of exorphins (structurally similar to endorphins) released from the partial digestion of the milk protein casein and the wheat protein gliadin[5]. They are potent psychosis-inducing factors[6].

Jyonouchi et al[7] suggested another mechanism for adverse effects of cow's milk protein (CMP) in autistic children. They stated that immune reactivity to dietary proteins including CMP may be associated with apparent dietary protein intolerance and gastrointestinal (GI) inflammation in ASD. This may be partly associated with an aberrant innate immune response against endotoxin, a product of the gut bacteria. Additionally, Jyonouchi et al[8] reported a positive association between TNF-alpha levels produced with endotoxins that stimulate innate immunity in the gut mucosa and those with CMP and its major components regardless of dietary interventions in ASD children who suffer GI symptoms. They concluded that intrinsic defects of innate immune responses are present in such children and this could offer an explanation to the link between GI and behavioral symptoms in ASD. Immune changes were reported in autistic children previously by Tomoum and associates[9].

This study was designed to assess the degree of benefit in various aspects of development of autistic children upon elimination of CMP from their diet and the possible correlation between CMP specific IgE and the degree of developmental delay in those patients.

METHODS

Subjects

The current study was conducted on 22 autistic children recruited from the Phoniatrics Unit and Psychiatric Outpatient Clinic, Ain Shams University Hospitals, Cairo, Egypt during the period from April, 2005 till March 2006. The recruited children fitted the criteria for diagnosis of autism according to the DSM IV[10]. Additionally, the diagnosis was confirmed and the patients were categorized based on their Childhood Autism Rating Scale (CARS) test score. They were 12 males and 10 females with an age range of 2.5 to 10 years (mean = 5.8 ± 2.4 years). The patients were compared to 30 healthy age and sex matched children enrolled as a control group. These were 17 males and 13 females with an age range of 2 to 8.5 years (mean = 5.9 ± 2.7 years).

After obtaining the approval of the local Ethical Committee at the Children's Hospital, Ain Shams University, each parent or caregiver gave an informed consent before participation in the study. We ensured that the enrolled autistic children were on no medications during the whole study period. The patients were randomly assigned to one of two groups. The parents or caregivers of the first group (11 patients, 6 males and 5 females with an age range of 3 to 10 years) were instructed to eliminate CM from the diet of their children throughout the study period while those of the second group were allowed to feed their children without restrictions. The second group included 11 autistic patients, 6 males and 5 females with an age range of 2.5 to 10 years.

Study measurements

Each patient was subjected to linguistic and developmental history taking as well as a comprehensive multidisciplinary complete psychiatric history taking. Dietetic history was also taken with a 24 hours recall of feeding to ensure that all autistic children met their recommended daily dietary allowances especially for calcium. Anthropometric measurements were taken and compared to the normal percentiles of growth[11]. Clinical examination was performed to detect any clinical evidence of atopic disease. CARS test was applied to ascertain the diagnosis of autistic disorder and determine its degree and a child psychiatric evaluation was performed. Venous blood samples were then withdrawn for laboratory procedures. The patients were finally subjected to language, intelligent quotient (IQ), social and mental age assessment.

Follow up

The children who participated in this work received interventional individual language therapy sessions and behavioral therapy in a semi-structured play situation; both therapeutic modules were administered concomitantly for six months, twice/week regularly. Each session was 20 minutes long. Both the language and behavioral therapy sessions progressed through different stages including attention control, manual activities to build up cognitive abilities and inner language and training to improve the language aptitudes aiming at increasing the size of the passive and active vocabulary. Clinical reevaluation of the patients was done at the end of the six months follow up including repetition of the language, social, mental age and IQ assessment.

Proper growth rates occurred in both groups and the autistic patients were within normal percentiles for age and sex throughout the whole study period. No clinical evidence of any nutritional deficiency was noticed in patients of either group during the 6 months follow up.

Serum CM specific IgE assay
Blood was collected from each patient or control upon enrollment by venipuncture under complete aseptic condition and was allowed to clot. Serum was separated by centrifugation at room temperature at 1500 rpm for 10 min and was stored at -20ºC for later quantitative determination of specific IgE to CM antigen. All samples were assayed in the same setting using an enzyme immunoassay kit (R-Biopharm GmbH, Darmstadt, Germany).

Childhood Autism Rating Scale
Childhood Autism Rating Scale (CARS), as explained by Schopler et al[12] is a test for identification of autism. It gives an accurate diagnosis by the age of 3 years. This test can be used to determine the severity of autistic symptomatology and can thus be useful in its periodic monitoring[13]. CARS test consists of 15 items, each rated on a 4-point scale (may be extended to 7 points by insertion of intermediate points). The child may be rated between two descriptions by using rating of 1.5, 2.5 or 3.5.
The 15 items include;

1. Relating to people.
2. Imitation.
3. Emotional response.
4. Body use.
5. Object use.
6. Adaptation to change.
7. Visual response.
8. Listening response.
9. Taste, smell and touch response and use.
10. Fear or nervousness.
11. Verbal communication.
12. Non verbal communication.
13. Activity level.
14. Level and consistency of intellectual response.
15. General impressions.

The total score of the test can range from 15 to 60 points according to the severity of autism. The score can be categorized into: non-autistic (15-29 points) as grade 0, mild to moderately autistic (30-36 points) as grade 1 and severely autistic (37-60 points) as grade 2.

Language assessment
Standardized Arabic language test was used in the current study[14]. This test is used to evaluate the language age of the child from zero to 8 years by assessing the child's attention through observation, the receptive and expressive part of the sementics, the receptive and expressive part of the syntax, the prosody, the pragmatics and the phonology.

Social age determination
Social age was determined by the Vineland Social Maturity Scale of Doll[15]. This scale is applied to assess the social behavior and abilities of the children and is suitable for children from birth to 15 years.

Mental age determination
Mental age was assessed by Stanford-Binet test[16]. This test is used to measure the child's cognitive abilities. It is suitable for children aging from 2-16 years. The test has two items, the verbal and the performance and the test item is chosen according to the child's abilities. Intelligence quotient (IQ) is calculated by dividing the mental age by the chronological age multiplied by 100.

Statistical analysis
Statistical analysis of the results was done via a standard computer programs SPSS (version 10) and Statistica software package version 5 (Statsoft, Tulsa, OK, USA). Non-parametric data were verified by the Kolmogorov-Smirnov test. The numerical data were presented as mean $\pm$ SD as well as median and interquartile range (IQR). Student-t test was used for comparing parametric data and Mann-Whitney U tests for non-parametric data. Paired-t test was used to compare parametric values after follow up while Wilcoxon Signed Ranks test was employed for non-parametric ones. Numeric variables were also related by correlation coefficient tests. The relations were considered significant if the probability (p) values were less than 0.05.

RESULTS

Table (1) shows that the studied groups of autistic children were comparable in terms of initial CARS test results, language, social age, mental age and IQ ($p>0.05$). This table also demonstrates significantly lower CARS ($p<0.01$) and significantly higher language age ($p<0.05$) in the group that underwent CM elimination diet compared to the other group after 6 months follow up. Significantly higher rates of change of CARS test results, language, social age, mental age and IQ in the CM elimination diet group were also detected as compared to the other group by the end of the trial ($p<0.001$, $p<0.05$, $p<0.05$, $p<0.01$ and $p<0.05$ respectively).

A significant decrease in CARS test results in both groups of autistic children is displayed in table (2) yet more evident in the first group. Concerning the language age, only the first group showed significant improvement on follow up ($p = 0.01$ and $p>0.05$ respectively). Both the social and mental ages improved in the first group only ($p< 0.01$ and

p>0.05 respectively). Although the IQ values seemed higher after 6 months in the autistic patients who were on CM elimination diet, the difference did not reach statistical significance (p>0.05).

Figure (1) shows significantly higher specific IgE levels against CMP in the autistic patients compared to the controls. The CM specific IgE expression was comparable among both patient groups. The mean IgE values were 21.9 ± 20.4 IU/ml [median and IQR are 20.0 and 38.0] for the first group and 22.5 ± 25.1 IU/ml [median and IQR are 17.0 and 38.3] for the second group (z = - 0.46, p>0.05).

When we classified the autistic children into categories according to their CARS test results, no significant differences were detected initially ($\chi 2$ = 0.21, p>0.05). Nevertheless, statistical significance between the two groups was evident after the follow up period ($\chi 2$ = 7.07, p<0.05). More patients from the group that underwent CM elimination diet belong to categories 0 and 1 compared to categories 1 and 2 in the second group by the end of the trial. Additionally, tables (3) and (4) show that 45.5% of patients who were on CM elimination diet went one CARS category down compared to only 36.4% from the second group.

Table 1. The CARS test, mental age, social age and IQ of the two studied groups upon enrollment and after follow up.

	Group I Patients on elimination diet	**Group II Patients without diet elimination**	**t/z***	**p**
Initial CARS	36.6±3.3 [37.0(5.0)]	38.1±4.0 [38.5 (8.0)]	-0.99	>0.05
FU CARS	30.8±2.9 [30.0(4.5)]	35.6±3.6 [35.0 (7.0)]	-3.47	<0.01
ROC of CARS	15.7±2.9 [16.2 (4.6)]	6.6±3.8 [7.1 (6.2)]	-6.39	<0.001
Initial language	1.9±2.1 [1.0 (1.3)]	1.4±1.58 [0.8(0.3)]	-0.97*	>0.05
FU language	2.4±1.9 [2.0(2.0)]	1.5±1.6 [1.0(0.3)]	-2.19*	<0.05
ROC of language	49.9±39.6 [33.3(100.0)]	7.6±17.7 [0.0(0.0)]	-2.50*	<0.05
Initial MA	2.2±1.0 [2.2(2.0)]	2.6±1.4 [2.0(2.0)]	-0.72	>0.05
FU MA	2.4±1.1 [2.5 (1.8)]	2.6±1.5 [2.0(3.2)]	-0.35	>0.05
ROC of MA	10.3±14.4 [8.3 (14.3)]	0.5±12.4 [0.0(10.1)]	-2.48*	<0.05
Initial SA	2.3±1.0 [2.5 (2.1)]	3.0±1.5 [2.3(3.3)]	-1.26	>0.05
FU SA	2.6 ±1.0 [2.9 (2.1)]	3.1±1.6 [2.4 (3.3)]	-0.59	>0.05
ROC of SA	15.0±8.4 [11.3(7.7)]	3.3±8.9 [0.0(7.6)]	3.17	<0.01
Initial IQ	37.9±9.1[38.0 (12.0)]	37.2±10.1 [38.0(15.0)]	0.18	>0.05
FU IQ	39.4±9.9 [40.0 (15.0)]	37.0±13.2[35.0(18.0)]	0.48	>0.05
ROC of IQ	4.0±11.4 [3.8(5.3)]	2.4±10.4[0.0(10.5)]	-1.92*	<0.05

*Non-parametric data as detected by Kolmogorov-Smirnov test. Test of significance used is Mann Whitney test. Data are presented as mean ± SD [median (IQR)].
CARS: childhood autism rating scale; FU: follow up; IQ: intelligence quotient; MA; mental age; ROC: rate of change; SA: social age.

Table 2. Comparison between the initial and the follow up CARS test, mental age, social age and IQ in each studied group.

	Group I Patients on elimination diet			**Group II Patients without diet elimination**		
	Initial	**Follow up**	**t/z* (p)**	**Initial**	**Follow up**	**t/z* (p)**
CARS	36.6±3.3 [37.0(5.0)]	30.8±2.9 [30.0(4.5)]	15.05 (p<0.001)	38.1±4.0 [38.5(8.0)]	35.6±3.6 [35.0(7.0)]	5.60 (p<0.001)
Language	1.9±2.1 [1.0(1.3)]	2.4±1.9 [2.0(2.0)]	-2.59* (p=0.01)	1.4±1.6 [0.8(0.3)]	1.5±1.6 [1.0(0.3)]	-1.34* (p>0.05)
MA	2.2±1.0 [2.2(2.0)]	2.4±1.1 [2.5(1.8)]	-3.40 (p<0.01)	2.6±1.4 [2.0(2.0)]	2.6±1.5 [2.0(3.2)]	0.26 (p>0.05)
SA	2.3±1.0 [2.5(2.1)]	2.6±1.0 [2.9(2.1)]	-2.97* (p<0.01)	3.0±1.5 [2.3(3.3)]	3.1±1.6 [2.4(3.3)]	-1.48* (p>0.05)
IQ	37.9±9.0 [38.0(12.0)]	39.4±9.9 [40.0(15.0)]	-1.29 (p>0.05)	37.2±10.1 [38.0(15.0)]	37.0±13.2 [35.0(18.0)]	0.14 (p>0.05)

*Non-parametric data as detected by Kolmogorov-Smirnov test. Test of significance used is Wilcoxon Signed Ranks test. Data are presented as mean ± SD [median (IQR)].
CARS: childhood autism rating scale; IQ: intelligence quotient; MA: mental age; SA: social age.

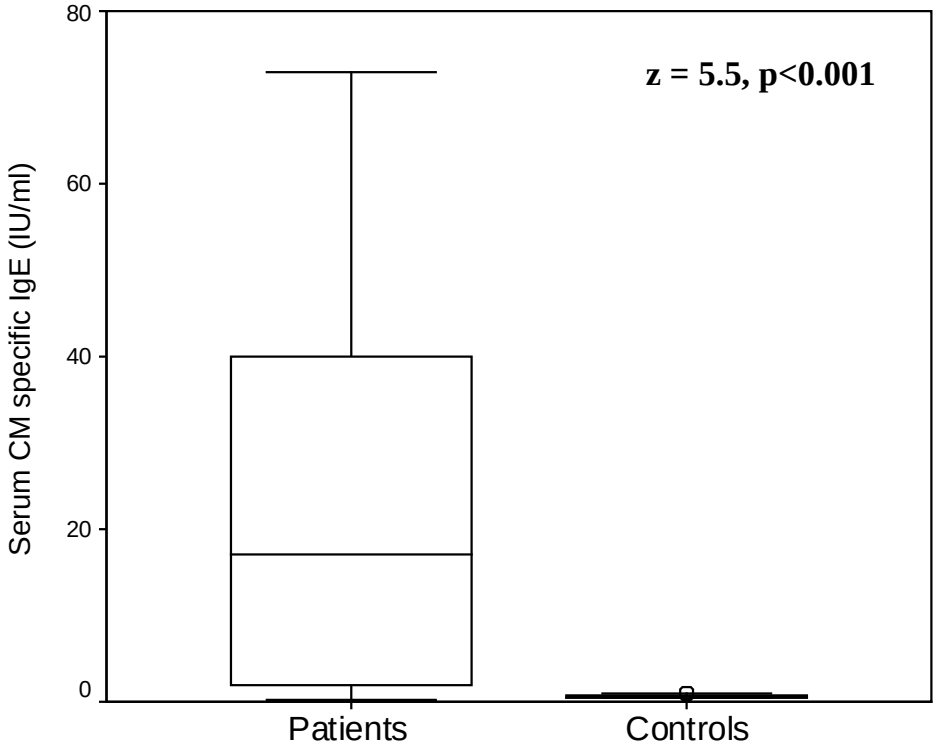

Figure 1. Serum CM specific IgE level in autistic children compared to the controls.

The boxes enclose the interquartile ranges (IQR) which are between the 25th and the 75th percentiles. The horizontal line inside the box represents the median and the whiskers represent the non outliner or extreme maximum and minimum values. The closed small squares represent extreme values (more than 3 IQR) and small open circles represent the outlier values (between 1.5 and 3 IQR).

Table 3. Changes in CARS categories upon follow up of the patients on CM elimination diet.

			CARS Category after Follow up (CARSA)		**Total**
			0	**1**	
Initial CARS Category (CARSB)	**1**	**Count**	3		3
		% within CARSB	100.0%		100.0%
		% within CARSA	100.0%		27.3%
	2	**Count**		8	8
		% within CARSB		100.0%	100.0%
		% within CARSA		100.0%	72.7%
Total		**Count**	3	8	11
		% within CARSB	27.3%	72.7%	100.0%
		% within CARSA	100.0%	100.0%	100.0%

CARS: childhood autism rating scale.

Table 4. Changes in CARS categories upon follow up of the patients who were not on CM elimination diet.

			CARS Category after Follow up (CARSA)		Total
			1	2	
Initial CARS Category (CARSB)	1	Count	4		4
		% within CARSB	100.0%		100.0%
		% within CARSA	57.1%		36.4%
	2	Count	3	4	7
		% within CARSB	42.9%	57.1%	100.0%
		% within CARSA	42.9%	100.0%	63.6%
Total		Count	7	4	11
		% within CARSB	63.6%	36.4%	100.0%
		% within CARSA	100.0%	100.0%	100.0%

CARS: childhood autism rating scale.

DISCUSSION

The significant improvement in the autistic children who were subjected to CM elimination diet as demonstrated by a significantly higher rate of change of CARS test results, language, social age, mental age and IQ compared to the rest of the studied sample enforces the potential adverse role of CM proteins in the etiopathogenesis of autism or at least in the progression of clinical severity. The use of an elimination diet to treat autism was previously demonstrated by Garvey[17], who weighed the potential benefits against some of the practical difficulties of keeping to a strict exclusion diet. The improvement on CM elimination in our series agrees with Lucarelli et al[4] who noticed a marked improvement in the behavioral symptoms of 36 autistic patients after a period of 8 weeks on CM free diet. Yet, this study included elimination of other foods which gave positive skin test results in their series.

Dohan[18] hypothesized that there is a defect in the intestinal barrier of schizophrenic patients that allows passage of neuroactive peptides of food origin into the blood and then into the cerebrospinal fluid to interfere directly with the CNS function. This hypothesis may be applicable to autistic patients as well because one member of the family of casomorphins, ß-casomorphins-7, is reported to be elevated in the urine of autistic patients[19]. From this toxic theory we shift to an immune theory suggested by Vojdani and colleagues[20]. They explained a mechanism by which bacterial infections and milk antigens may modulate autoimmune responses in autism. In their series, autistic children showed the highest levels of IgG, IgM and IgA auto-antibodies against nine different neuron-specific antigens as well as three cross-reactive peptides including milk protein. They suggested that these antibodies may have been synthesized as a result of an alteration in the blood-brain barrier. This barrier failure promotes access of preexisting T-cells and CNS antigens to immunocompetent cells, which may start a vicious cycle.

The current study revealed significantly higher IgE against CMP in autistic children compared to the controls. This finding comes in agreement with Lucarelli et al[4] and it further emphasizes the role of the immune system in the occurrence of adverse reactions to CMP in ASD children. Additionally, Ashwood and coworkers[21] reported diffuse mucosal immunopathology in some ASD children and highlighted the potential for benefit from dietary therapies. Further studies that include oral food challenges with CM are needed to verify the role of allergy or intolerance in this context.

In conclusion, autistic children showed improvement in language, cognition and behavioral capabilities upon CM elimination and they demonstrated high levels of specific IgE antibodies against CMP suggesting an allergic element in the pathogenesis of this potential adverse reaction to CM. The findings are limited by the sample size

and further trials are indicated. However, our data re-enforce the concept of involvement of CM in the etiology or at least the progression of autistic features. We recommend trials of CM elimination diet and re-challenge in autistic children with replacement by safe alternatives hoping for better achievement during their interventional programs.

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