Gastrointestinal disturbances and immune response to gluten in Pervasive Developmental disorders

Prof.Ghada El Dory, Professor Of Paediatrics, Department Of Medical Study Institute Of Postgraduate Childhood Studies Ain Shams University
Prof.Hanaa Hamdy Ahmed, Professor Of Biochemistry, Hormonal Department, National Research Center
Prof.Ehab Mohamed Eid, Professor Of Public Health, Department Of Medical Study, Institute Of Postgraduate Childhood Studies Ain Shams University
Dr.Inas Refaei El-Aleemy, Ass.Prof Of Paediatrics Child Health Department National Research Center
Manal Gamil Ahmed Sakr, M.Sc. of Paediatrics

Abstract

Objectives: Gastrointestinal symptoms are a common feature in children with pervasive developmental disorders, drawing attention to a potential association with celiac disease or gluten sensitivity. However, studies to date regarding the immune response to gluten in Pervasive Developmental disorders and its association with celiac disease have been inconsistent.

Subjects and Methods: This cross sectional case control study included 45 patients aged 3 to 12 years (with or without gastrointestinal symptoms) diagnosed with Pervasive Developmental Disorders according to DSM- IV TR, Childhood Autism Rating Scale (CARS) and Gallium test for autistic characters. EEG was done to diagnose epilepsy. They had been regularly attending out patient clinic of center for care of children with special needs, institute of postgraduate childhood studies; Ain shams University, Egypt for at least one year. Forty five apparently healthy children of matched age and sex were recruited as a control group. Serum levels of IgG, IgA, IgM class antibodies to gliadin were measured by using ELISA methods.

Results: A total of forty five autistic children with confirmed diagnosis aged between 3 to 12 years were studied. They were 36 males and 9 females with male to female ratio 3.5:1. The mean age of introduction to cereals was 6 months (range 4- 8 months). The main gastrointestinal symptoms as abdominal distension was present in 20 patients (44.4%), constipation in 16 patients (35.6%), chronic diarrhea in 8 patients (17.8%), vomiting in 9 patients (20%), anorexia in 19 patients (42.2%), iron- deficiency anemia that does not respond to iron therapy in 24 patients (53.3%), feeding difficulties in 10 patients (22.2%). None of the autistics examined were positive for IgA and IgG antibodies tested, and 60% patients showed high serum levels of IgM antibodies to gliadin.

Conclusion: The increased anti- gliadin antibody response and its association with GI symptoms points to a potential mechanism involving immunologic and/or intestinal permeability abnormalities in affected children. Immunological detection of, IgA, IgM and IgG antibodies class to gliadin are useful tool in the diagnosis and follow- up of the disease.

انتشار الاضطرابات المعدية المعوية والاستجابة المناعية للجليوتين فى الإضطرابات النمو الارتقائى الشاملة

الأهداف: أعراض معدية معوية هى سمة مشتركة فى الأطفال الذين يعانون من اضطرابات النمو الارتقائى الشاملة، لافته الانتباه إلى جمعية محتملة مع مرض الاضطرابات الهضمية أو الحساسية للجليوتين.

المواد وطرق: تم رسم مخ لتشخيص الصرع. كانوا يحضرون بانتظام عيادة المرضى من مركز لرعاية الأطفال ذوى الاحتياجات الخاصة، معهد الدراسات العليا للطفولة. جامعة عين شمس، مصر لمدة عام واحد على الأقل. تم تجنيد خمسة واربعون طفلا اصحاء من العمر يقابل والجنس كمجموعة تحكم. مستويات المصل من الأجسام المضادة المناعي للجليادين تم قياسها باستخدام أساليب ELISA.

النتائج: تمت دراسة ما مجموعه خمسة وأربعين الأطفال الذين يعانون من التوحد مع تأكيد التشخيص الذين تتراوح أعمارهم بين ٣إلى ١٢ سنوات. كانو ٣٦ ذكور و٩ إناث مع نسبة الذكور إلى الإناث ٣,٥– ١. تم تسليم جميع المرضى فى فترة ولاية كاملة من خلال الولادة المهبلية الطبيعية. وكان متوسط عمر مقدمة الحبوب ٦ أشهر (المدى ٤– ٨ أشهر). وكان متوسط العمر الذى بدأت أعراض ٨ أشهر، مجموعة ٤– ١٢ شهرا. وكان النمو وتأخر فى النمو الحالى فى ٥ مرضى (١١,١%). كان القلق موجودا ٢٢,٢% من المرضى، ومشاكل النوم فى ١٣,٣%، والاكتئاب في ١١,١%، وفرط النشاط في١١,١%، والسلوك المضر بالنفس فى ٤,٤%. وكانت الأعراض المعدية المعوية الرئيسية على النحو انتفاخ البطن الحالية فى ٢٠ مريضا ٤٤,٤%)، والإمساك فى ١٦ مريض (٣٥,٥%)، والإسهال المزمن فى ٨ المرضى (١٧,٨%)، والتقيؤ فى ٩ مرضى (٢٠%)، وفقدان الشهية فى ١٩ مريضا (٤٢,٢%)، وفقر الدم الناتج عن نقص الحديد الذى لا يستجيب للعلاج الحديد فى ٢٤ مريضا (٥٣,٣%)، صعوبات والتغذية فى ١٠ مريضا (٢٢,٢%). ولم يكن أى من المتوحدون فحص إيجابى لإعتلال وIgG اختبارها، وأظهر ٦٠% من المرضى مستويات المصل عالية من الأجسام المضادة جليوتين المناعى لجليادين. وأظهر المرضى الخاضعين للدراسة لدينا مستويات أعلى كبيرة من تلك الأجسام المضادة فى مصل الدم.

الخلاصة: الأطفال الذين يعانون من الإضطرابات النمو الارتقائى الشاملة يعرض زيادة التفاعل المناعى لجليوتين وآلية التى يظهر لتكون متميزة عن أنه فى مرض الاضطرابات الهضمية. زيادة مكافحة لجليادين استجابة الأجسام المضادة وارتباطه أعراض الجهاز الهضمى نقطة لآلية المحتملة التى تنطوى على تشوهات نفاذية المناعية و/ أو المعوية عند الأطفال المتضررين. كشف المناعى الكازين،IgA, IgMوIgG فئة لجليادين هم أداة مفيدة فى تشخيص ومتابعة المرض.

Introduction:

Pervasive developmental disorders are behavioral disorders with onset before 36 months characterized by impairment of social interest and behaviors. Other characteristics include, sensory dysfunction, in appropriate laughing arid giggling little or no eye contact, apparent insensitivity to pain, preference to be alone and many more according to the American psychiatric association (American psychiatric Association 1994).

In the last 20 years, an increase in the incidence of pervasive developmental disorders has unexplained. The etiology of PDDs is complex and usually, the underlying pathologic mechanisms are unknown. Some scientists emphasize the possible impact of a number of postnatal factors ranging from environmental toxins to dietary factors (Waterhouse et.al., 1996).

Gastrointestinal diseases are more common in children with neurological disability (Sullivan, 1997, and Torrente et.al., 2002) describe unexpected intestinal inflammation with low grade colitis. Wakefield et.al. (2002) have suggested that peptides formed through the incomplete breakdown of foods containing gluten and casein derived from dairy product, exhibit direct opiod activity or form ligands for peptidase, which break down endogenous endorphins and enkephalins. Individuals who cannot metabolize gluten produce antigliadin which they can not metabolize further. This A gliadin binds to A and D opoid receptors. These receptors associate with mood and behavior.

Opoids like gliadorphin (gluten opoid and casein opiod) are toxic for children with pervasive developmental disorder due to the fact that these children have abnormal leaky gastrointestinal tract. Instead of completely digesting and excreting these opioid proteins, some of the partially digested gluten and casein proteins leak out of the gut and are transported to other parts of the body before they can be completely digested. The opoid protein travels through the bloodstream cross the blood brain barrier to enter brain and stimulate morphine like effect casein protein and causing inattentiveness, unclear thinking, irregular sleeping and eating patterns (Shattock and Whiteley, 2002).

Gastrointestinal symptoms are a common feature in children with pervasive developmental disorders, drawing attention to a potential association with celiac disease. Eliminating gluten and casein has shown to be effective in reducing some of the behavioral symptoms of autism. Knivsberg et.al, (1995) looked at the neurological effects of the dietary gluten fraction gliadin in autistic children who had gastrointestinal problems. When the children were exposed to an oral dose (1 g) of gliadin, frontal nerve impulses were significantly inhibited.

Johnson and Myers (2007) were noted an improvement of social, cognitive, and communication skills when they were placed on a diet free of gluten. Therefore, the present study aimed at assessing the prevalence of gastrointestinal disturbances in pervasive developmental disorders, and studying the determinants of gastrointestinal disturbances and pervasive developmental disorders represented in anti- gliadin antibody (IgA, IgM and IgG) and evaluating the potential link between Pervasive Developmental disorders and gastrointestinal disturbances.

Patients And Methods

Patients:

This cross sectional case control study included 45 patients aged 3 to 12 years (with or without gastrointestinal symptoms) diagnosed with Pervasive Developmental Disorders. The inclusion criteria for selection included all children with Pervasive Developmental Disorders and gastrointestinal symptoms, who had been regularly attending out patient clinic of center for care of children with special needs, institute of postgraduate childhood studies; Ain shams University, Egypt for at least one year. Both males and females were included. Exclusion criteria included autistic children suffering from acute or chronic infection; Drugs affecting gastrointestinal tract. The diagnosis of Pervasive Developmental Disorders was established on the basis of medical history, physical examination according to DSM- IV TR, Childhood Autism Rating Scale (C.A.R.S.) and Gallium test for autistic characters. EEG was done to diagnose epilepsy. The parents were asked to complete a questionnaire regarding the child's medical and behavioral history. Control group included forty five age, sex and social class comparable apparently healthy children without history suggestive of medical, neurological or psychiatric disorders. They were 10 males representing (50%), and 10 females representing (50%). They were selected from the outpatients' clinic at National Research Center while they were coming for follow up. The study was conducted in accordance to ethical procedures of institute of postgraduate childhood studies, Ain Shams University, and National Research Center (NRC) ethics. Written informed consent was obtained from the parents of the participating children.

Methods:

All patients will be subjected to full detailed history including age and sex of patients, perinatal, developmental, immunization history, and history of behavior disorders, the presence of convulsions, duration and course of disease, age of beginning of treatment and the treatment regimen, co morbidity or complications. Inquiring about special diets, a prior of food allergy, types of food selectivity, frequent vomiting, a prior diagnosis of gastro esophageal reflux, abdominal pain, abnormal stool pattern, characteristics of the bowel movements, diarrhea, constipation, use of laxatives or enemas, fecal soiling, number of bowel movements per day and prior visits to a gastroenterologist. Through clinical examination including general and, systemic examination (chest, heart, abdomen), and neurological evaluation. The severity of gastrointestinal disturbance will be evaluated. The parents were asked to complete a questionnaire regarding the child's medical and behavioral history.

Biochemical Measurements

From all cases, and controls, 5 cc venous blood samples were obtained by venipuncture and collected in plain tubes, and after gentle mixing allowed to clot at room temperature, and then centrifuged for 10 minutes. Serum Human Casein antibody level was measured using an enzyme-

linked immunosorbent assay (ELISA) kit (Glory science, USA) according to the method described by Greenberg (14). The kit use a double- antibody sandwish enzyme- linked immunosorbent assays (ELISA) to assay the level of human casein in samples. All wash steps were performed using an ELISA washer (Robonik ELISA plate washer, Mahape, Navi Mumbai, India Biotek EL×800 Mumbai, India), whereas the absorbance of all samples were read using the ELISA reader (Biotek EL×800) at 450 nm. A standard curve of the absorbance versus concentration was plotted using the calibrators. The concentration of Casein antibody level in the samples was determined directly from the curve. Cutoff values for serum Human Casein <200 ng/mL is considered to be normal.

Samples were assayed in a single large batch, in duplicate. Anti-gliadin antibody (IgA, IgM and IgG) was measured by an enzyme- linked immunosorbent assay using (ELISA) kit (Immunospec corporation, CA) according to the method of Trocone and Ferguson (1991). The immunospec gliadin IgA, IgG, IgM ELISA test system is designed to detect IgA, IgG, IgM class antibodies to Gliadin in human sera. Walls of plastic microwell strips are sensitized by passive absorption with Gliadin antigen. The test procedure involves three incubation steps: test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the Immobilized antigen. The plate is washed to remove unbound antibody and other serum components and peroxidase conjugated goat anti- human IgA, IgG, IgM in chain specific is added to the wells and the plate is incubated, and the conjugate will react gliadin antibody Immobilized on the solid phase in step 1. The wells are washed to remove un- reached conjugate. The microwells containing immobilized peroxidase conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxides produces a color change. After a period of time the reaction is stopped and the color Intensity of the solution is measured photometrically. The color Intensity of the solution depends upon the antibody concentration in lie original test sample. The plates were read at a wavelength of 450nm and measure the optical density (OD) of each wall against the reagent blank. The plate was read within 30 minutes after the addition of the Stop Solution. The mean OD of the Calibrator was calculated. A cutoff OD value (0.39) for positive samples has been determined by the manufacturer and correlated to the Calibrator. The correction factor was determined, and the cutoff OD value was calculated by multiply the CF by the mean OD of the Calibrator determined above.

Statistical Analysis:

Data obtained from the research will be organized, tabulated and analyzed through IBM personal computer. Statistical analyses were performed using the SPSS statistical package software for Windows version 20 (SPSS Inc., Chicago, Illinois, USA). Parametric variables are expressed as the mean ± SD. Differences between parametric variables among the controls and the studied patients groups were analyzed using two tailed unpaired t- test. Qualitative variables were assessed by Chi-square test. A P value <0.05 was considered significant difference and p< 0.005 was considered highly significant difference.

Results:

A total of Forty five children with pervasive developmental disorders aged between 3 to 12 years were studied. They were 36 males and 9 females with male to female ratio 3.5:1. All patients had been delivered at full- term through normal vaginal delivery. Growth and developmental delay were present in 5 patients (11.1%). Table (1) illustrates Socio-Demographic and clinical data of the studied cases. Anxiety was present in 22.2% patients, sleep problems in 13.3% patients, depression in 11.1% patients, hyperactivity in 11.1% patients, and self injurious behavior in 4.4% patients. Figure (1) showed behavior disorders of the studied patients.

In this study, 21 patients (48%) had perinatal complications in the form of asphyxia (25.7%), jaundice in (11.9%), head trauma in (2.2%) neonatal convulsions in (2.2%), and respiratory distress in (4.4%).

The main gastrointestinal symptoms of the patients suffering from pervasive developmental disorders were abdominal distension in 20 patients (44.4%), constipation in 16 patients (35.6%), chronic diarrhea in 8 patients (17.8%), vomiting in 9 patients (20%), anorexia in 19 patients (42.2%), iron- deficiency anemia that does not respond to oral iron therapy in 24 patients (53.3%), and feeding difficulties in 10 patients (22.2%). Table (2) showed gastrointestinal symptoms of the studied patients.

Our studied patients with autism showed statistically significantly higher levels of serum (IgM) class antibodies to gliadin, serum casein antibodies, and significantly lower serum levels of DPP- IV (P<0.000 in all). None of the autistics examined were positive for IgA and IgG antibodies tested, and 60% patients showed high serum levels of IgM antibodies to gliadin. Table (3) showed comparison of the laboratory findings of the studied patients and control groups. Table (4) showed the percentages of serum antigliadin IgA antibodies in the studied patients. Figure (2) showed comparison of serum levels of IgA- AgA, and IgG-AgA of the studied patients versus control groups. Figure (3) showed comparison of serum levels of IgM- AgA of the studied patients versus control groups.

There are significant relationships between serum levels of IgA, IgG, IgM antigliadins antibodies, casein antibody and DPP- IV activity with some GIT symptoms. Table (5) showed relation of serum levels of IgA, IgG, IgM antigliadins antibodies with some GIT symptoms in the studied cases group.

Discussion:

Gastrointestinal diseases are more common in children with a neurological disability and previous reports describe unexpected intestinal inflammation, with low- grade colitis, (Torrente et.al. 2002& Melmed et.al. 2000) and duodenitis with reduced disaccharides in children with autism. Immunohistochemical studies suggest an immune response targeted at the gut epithelium, with a possible autoimmune cause (Murch et.al. 1998& Dalrymple et.al. 1992). Immunologic hypersensitivity to the

gliadin component of wheat and other cereal grains causes gluten-sensitive enteropathy. This results in malabsorption and fatty diarrhea. The consequences of malabsorption are weight loss, nutritional deficiencies, and growth failure in children (Papadopoulou et.al. 1994). The gastrointestinal (GI) lesion responds rapidly to gluten exclusion from the diet, both clinically and histologically (Lucarelli et.al. 1995).

Knivsberg et.al. (2001), claimed that gluten sensitivity could play an important role in the pathophysiology of autism. Knivsberg et.al. 2002 have suggested that food peptides derived from gluten and casein might be able to determine toxic effects at the level of the central nervous system by affecting neurotransmitter releases, uptake and metabolism and behavioral consequences.

Therefore, the aim of our study is to assess the prevalence of gastrointestinal disturbances in pervasive developmental disorders, and to study determinants of gastrointestinal disturbances and pervasive developmental disorders through the measurement of casein antibody, and anti- gliadin antibody (IgA, IgM and IgG) levels and to evaluate the potential link between Pervasive Developmental disorders and gastrointestinal disturbances.

This cross sectional case control study included 2 groups of children, forty five patients in group I with Pervasive Developmental Disorders and gastrointestinal symptoms. They were diagnosed with Pervasive Developmental Disorders according to DSM- IV TR, Childhood Autism Rating Scale (C.A.R.S.) and Gallium test for autistic characters. They had been regularly attending out patient clinic of center for care of children with special needs, institute of postgraduate childhood studies; Ain shams University, Egypt for at least one year. Forty five apparently healthy children from public schools of matched age and sex were recruited as a control group with no history of pervasive developmental disorders in group II.

In our present study, the studied patients' age was ranging between (3-12) years, (36 male and 9 female) with male to female ratio 3.5:1. This was in accordance with (Luke& Tsai, 2004) that has shown a predominance of boys over girls. Ratio of 3 or 4 boys to 1 girl have consistently been reported.

It is hypothesized that males have lower threshold for brain dysfunction than females, resulting in a higher incidence of autism in males. According to this hypothesis more severe brain damage would be required to produce autism in a girl, resulting in more severely impaired autistic child (Rapin, 2002). In our present study, Girls are usually more severely affected than boys and on an average score less on intelligence tests.

In our present study, all patients had been delivered at full- term through normal vaginal delivery, and there was no history of bleeding after the first trimester and maternal use of medication, prenatal infections, or obstetric complications. It is not clear whether obstetric complications caused PDDs or whether PDDs& obstetric complications resulted from another problem (James, 2005). The results of numerous studies show the many PDDS children have organization disorders such as cerebral palsy, hydrocephalus, congenital rubella, toxoplasmosis, tuberous sclerosis, cytomegalovirus infection, meningitis, encephalitis, severe brain hemorrhage and many types of epilepsy. (Tasi, 2004) has reported that other predisposing factors appear in children with the histories of pervasive developmental disorders. These predisposing factors may include increased maternal age, bleeding after the first trimester and maternal use of medication.

In our present study, 21 patients (48%) had perinatal complications in the form of asphyxia (25.7%), jaundice in (11.9%), head trauma in (2.2%) neonatal convulsions in (2.2%), and respiratory distress in (4.4%). This is in the agreement with a number of studies that have shown an increased frequency of perinatal and neonatal complications in PPDs. PPDs have significantly more congenital anomalies than do in siblings that suggest the complication in the first trimester are more common (Burd et.al., 1999). A higher than expected incidence of perinatal complications seems to occur in infants who are later diagnosed with autistic disorder in the neonatal period autistic children has a high incidence respiratory distress syndrome and neonatal anemia (Kaplan& Sadoct, 2004). Kernicteras, which describes basal ganglia damage secondary to neonatal jaundice, was also reported to PPDs autistic disorders. This can be explained in the light of the increasing evidence that the volume and function in the basal ganglia are different in PDDs (Karnebeek et.al. 2002).

In a study by Schultz et.al (2006) carried on 861 children with autistic disorders, and 123 control children, Who reported that, children who were not breast fed or were fed infant formula without doeosahexaonoic acid arachiolonic supplementation are significantly more likely to have pervasive developmental disorder. In our present study, (56.7%) patients were breast fed and (43.3%) patients were artificial fed.

In this study 18 out of 45 Children (40%) had epilepsy. This result coincided with that reported by Muroz et.al. (2008), who reported that the rate of epilepsy in PDDs is higher than in other developmental disorders and estimates point to frequency range between 7% and 42%. Ashwood& Van de water, (2004) and Vojdani et.al, (2004) who concluded that a subgroup of patients with PDDs most of them autistic Disorder produce antibodies against purkinge cells and gliadin and casein peptides which may be responsible for some of the neurological symptoms in autism. Epilepsy occurs in up to 30 percent of those with autism can amplify their symptoms Murphy (2001).

The mechanical production of speech is impaired in autistic patients. The speech may be like that of robot there may be chanting or singsong speech, with odd prolongation of sound syllables and words odd respiratory rhythms may produce staccato speech in some PDDS individuals& (Luke& Tsai, 2004).

In this study, behavior disorders such as anxiety was present in 9 patients (20%), depression in 5 patients (11.1%); Aversion to particular sounds in 10 patients (22.2%), angry outbursts in 8 patients (17.8%) self injurious behavior in 2 patients (4.4%), sleep problems in 6 patients

(13.3%), and hyperactivity in 5 patients (11.1%). Consistent with Luke and Tsai, 2004, the effective expression of PDDs people may affect their mood often is labile Sobbing, crying, or screaming may be unexplained. In appropriate laughing and giggling may occur for no obvious reason. Peculiar habits such as hair pulling, biting parts of body were present particularly in mentally retarded PDDs children.

Research has revealed that PPDs autism has familial links with other mental disorders, notably depression, obsessive- compulsive disorders and motor tics. Depression is more frequent in immediate relatives and pre-dates the arrival of the child with PDDs autism. However, it occurrence is linked to the development of depressions in the child with PDDs Murphy (2001).

Children with pervasive developmental disorders frequently develop gastrointestinal symptoms such as constipation, diarrhea, abdominal discomfort and distension (Reichelt et.al. 1990). In our present study, the main GIT symptoms as abdominal distension were present in 20 patients (44.4%), constipation in 16 patients (35.6%), chronic diarrhea in 8 patients (17.8%), vomiting in 9 patients (20%), Anorexia in 19 patients (42.2%), iron- deficiency anemia that does not respond to iron therapy in 24 patients (53.3%), feeding difficulties in 10 patients (22.2%). Growth and developmental delay were present in 5 patients (11.1%). This is in the agreement with Horvath et.al, 1999, who reported a 69% prevalence of histological esophagitis and a 58% prevalence of intestinal disaccharides deficiency in a group of 36 autistic children studied by upper gastrointestinal endoscopy and biopsy.

Constipation is a relatively common problem in children, with estimates of prevalence in otherwise, normal children reaching up to 8% and 30% in autistic children. The significantly higher incidence of constipation observed in autistic children examined in this study, could be due to the fact that they are very meticulous eaters with eating problems, most of them refusing to eat fruits and vegetables. Some scientists reported an improvement in social interaction, communication and imaginative skills in autistic children on gluten free diet as compared to control (Ciclitira et.al. 2001).

Consistent with Ghaem et.al. (1998), our present study showed that children with autistic disorders frequently have reflux esophagitis. Infants and children with gastroesophagal reflux disease more frequently have sleep disturbance than the normal population. Night time wake- up with pain, abdominal discomfort or both is common feature of gastroesophageal reflux and reflux esophagitis in children. There is higher prevalence of sleep disturbances and sudden irritability in children with PDDS who had GI symptoms.

Our studied patients with autism showed statistically significantly higher serum levels of (IgM) class antibodies to gliadin, and serum casein antibodies compared with unrelated healthy controls, ($P<0.05$ in all). None of the autistics examined were positive for IgA and IgG antibodies tested, and 28 patients (62.2%) showed high serum levels of IgM antibodies to gliadin. This is in the agreement with Al- Ayadhi (2006), who reported that, none of the autistic examined were positive for any of the antibodies tested, including anti- gliadin antibody (IgA and IgG). However, our results are not in the agreement with Trajkouski (2008), who found elevated levels of antibodies to gluten and casein in population of 35 autistic children relative to 21 of the subjects' neurotypical sibiling. Antigliadin antibodies are produced against gluten present in wheat, they are used to diagnose gluten sensitive entropathy, can be found in autism. Kawashati, et.al. (2006) performed IgG- AGA testing for gluten and casein in 30 children with autism 53% were observed to be positive for casein, while 50% were positive for glutens.

In this study, there are significant relationships between serum levels of IgA, IgG, IgM antigliadins antibodies, and some GIT symptoms. This is in accordance with the results of the study conducted by Nicolov et.al. (2009).

Conclusion:

In the children with autism, serum levels of anti- gliadin IgM antibody were higher compared to healthy controls. The increased anti- gliadin antibody response and its association with GI symptoms points to a potential mechanism involving immunologic and/ or intestinal permeability abnormalities in affected children. This research supports parents trying a gluten- free diet for their child with autism. Studies with larger sample sizes are needed to confirm the results obtained in this study.

References:

1. Al- Ayadhi LY (2006): Gluten sensitivity in autistic children in Central Saudi Arabia. **Neurosciences** (Riyadh). Jan; 11 (1):1 1- 14.
2. American Psychiatric Association (APA) (1994): **Diagnostic and Statistical Manual of mental disorders 4th edition (DSM IV)**. Washington DC: APA.
3. Burd L, Severud R, Kerbishian J, et.al (1999): Prenatal and perinatal risk factors of autism. **J Perinat Med**, 27: 441- 450.
4. Dalrymple NJ, Ruble LA (1992): Toilet training and behaviors of people with autism: parent views. **J Autism Dev Disord** 1992; 22: 265- 275.
5. Horvath K, Papadimitriou JC, Rabsztyn A, Drachenberg C, Tildon JT (1999): G astrointestinal abnormalities in children with autistic disorder**. J Pediatr**; 135: 559- 563.
6. James R (2005): **Autism**, available at e- medicine website. Last updated May 23, 2005.
7. Johnson CP, Mayer SM (2007): council on children with disabilities" identifications and evaluation of children with autism spectrum disorders. **Pediatrics** 120: (5):1 183- 215.
8. Kaplan IL and Sadock B (2004): **Pervasive Developmental Disorders**. In: Kaplan and Sadock's synopsis of psychiatry: behavioral sciences. Clinical psychiatry, Lippincott Williams and Wikins publishing, Inc. 9th edition, 42: 1208- 1223.
9. Karnebeek CD, Gelderen I, Nijhof GI et.al (2002): An aetiological study of 25 mentally retarded adults with autism. **J Med Genet** 39:

205- 213.

10. Kawashti MI, Amin OR, Rowehy, NG (2006): Possible Immunological disorders in autism: concomitant autoimmunity and immune tolerance. **Egypt J Immunol**. 13 (1):9 9- 104
11. Knivsberg AM, Reichelt K, Nodland M, Hoien T (1995): Autistic Syndromes and Diet: A Follow- Up Study. **Scandinavian Journal of Educational Research**; 39: 223- 236.
12. Knivsberg AM, Reichelt KL, Hoien T, Nodland M (2002):A randomized, controlled study of dietary intervention in autistic syndromes. **Nutr Neurosci** 5: 251- 261.
13. Knivsberg AM, Reichelt KL, Nodland M (2001): Reports on dietary intervention in autistic disorders. **Nutr Neurosci** 2001; 4: 25- 37.
14. Lucarelli S, Frediani T, Zingoni AM (1995): Food allergy and infantile autism. **Panminerva Med** 1995; 37: 137- 141.
15. Luke Y and J Tsai (2004): **Autistic disorders. In text book of child and adolescent psychiatry**, Wiener JM& Dulcan MK, 3rd edition.
16. Melmed RD, Schneider CK, Fabes RA, Philips J, Reichelt K (2000): Metabolic markers and gastrointestinal symptoms in children with autism and related disorders [abstract**]. J Pediatr Gastroenterol Nutr** 31 (Suppl 2): S31- S32.
17. Murch S, Thomson M, Walker- Smith J (1998): Autism, inflammatory bowel disease, and MMR vaccine. **Lancet** 351: 908- 912.
18. Muroz YA, Palau D M (2008): Regression in autism. **Rev Neurol.** 46 Suppl; S 71- 7.
19. Murphy M, Cowan R and Sederer L (2001): disorders of Childhood and Adolescence. 2nd ed. **Blueprints in Psychiatry**; P 42. Malden, Mass: Blackwell Science, Inc.
20. Nikolov RN, Bearssk E, Lettinga J, Erickson C, Rodawski M, Aman MG,
21. McCrackean, McDougle, Tierney E, Vitiello B. Arnold LE, Shah B, Posey DJ (2009):G astrointestinal proplems in autistic children. **J Autism Dev Disord**, Mar, 3 (3):4 05- 13
22. Papadopoulou A, Clayden GS, Booth IW (1994): The clinical value of solid marker transit studies in childhood constipation and soiling. **Eur J Pediatr**; 153: 560- 564.
23. Rapin I (2002): **Diagnosis and management of autism**. In: Recent advances in pediatrics. New York publishing; 8: 121- 134.
24. Reichelt KL, Scott H, Ekrem J (1990): Gluten, milk proteins and autism: the results of dietary intervention on behavior and peptide secretion**. J Appl Nutr**; 42: 1- 11.
25. Shattock P and Whiteley P (2002): The Sunderland Protocol: A logical sequencing of biomedical intervention for the treatment of autism and related disorders. Sunderland, UK: autism Research Unit, University of Sunderland.
26. Sullivan PB (1997): Gastrointestinal problems in the neurologically impaired child. **Baillieres Clin Gastroenterol**; 11: 529- 546.
27. Torrente F, Ashwood P, Day R, Machado N, Furlano RI, Anthony A et.al. (2002): Small intestinal enteropathy with epithelial IgG and complement deposition in children with regressive autism. **Mol Psychiatry**; 7: 375- 382.
28. Trajkovski, V et.al (2008): Higher Plasma Concentration of Food-Specific Antibodies in Persons with Autistic Disorder in Comparison to Their Siblings. Focus on Autism and Other Developmental **Disabilities**, Vol. 23, No. 3:176- 185, 2008.
29. Tsai L (2004): Autistic disorder in: Textbook of child and adolescent psychiatry, the American psychiatric publishing, Inc. 3rd ed; 20: 261- 315.
30. Wakefield A, Puleston J, Montgomery S. et.al (2002): "The concept of entero- colonic encephalopathy, autism, and opioid receptor ligands". **Alimentary pharmacology and Therapeutics** 16, 663- 674.
31. Waterhouse L, Fein D, Modahl C (1996): Neurofunctional mechanisms in autism. **Psychol Rev;** 103: 457- 489.

Table (1) Socio- Demographic and clinical data of the studied cases

Variables (Present History)		No. (%)
Sex	Male	36 (80%)
	Female	9 (20%)
Handedness	Right	29 (64.4%)
	Left	9 (20%)
	Both	7 (15.6%)
	Delayed Growth	5 (11.1%)
	Delayed Speech	25 (55.6%)
	Developmental Delay	5 (11.1%)
	Age At Onset Of Symptoms	5 (11.1%)
	Hyperactivity	2 (4.4%
	No Eye To Eye Contact	3 (6.7%)
	Epilepsy	18 (40%)
	Otitis Media	16 (35.6%)
Postnatal Complications	Asphyxia	12 (26.7%)
	Jaundice	5 (11.9%)
	Head Trauma	1 (2.2%)
	Neonatal Convulsions	1 (2.2%)
	Respiratory Distress	2 (4.4%)
Sphincteric Control	Enuresis	14 (31.1%)
	Encorporesis	37 (82.2%)

Table (2) Gastrointestinal symptoms of the studied patients.

GIT Symptom	No. (%)
Vomiting	9 (20%)
constipation	16 (35.6%)
Diarrhea	8 (17.8%)
Abdominal Distension	20 (44.4%)
Past History Of Dehydration	10 (22.2%)
Recurrent Attacks Of Abdominal Pain	14 (31.1%)
Fatigue	11 (24.4%)
Failure To Thrive	13 (28.9%)
Iron- deficiency anemia that does not respond to iron therapy	24 (53.3%)
Food Sensitivity	19 (42.2%)
Intolerance Of Particular Foods	18 (40%)
Anorexia	19 (42.2%)
Feeding Difficulties	10 (22.2%)

Table (3) Comparison of the laboratory findings of the patients and control groups.

Variable	Case (n= 45)	Control (n= 20)	T Value	P
	Mean± SD	Mean± SD		
Serum Iga- Antigliadin Antibody	95.67±15.89	161.60± 23.65	13.21	<0.01
Serum Igg- Antigliadin Antibody	90.20± 80.73	153.02± 31.61	3.35	<0.05
Serum Igm- Antigliadin Antibody	2.74± 1.21	0.96± 0.19	9.64	<0.01

Table (4) Percentages of serum antigliadin IgA antibodies in the studied patients.

Variable	N	%
Normal Antigliadin Iga	45	100
Normal Antigliadin Igg	45	100
Negative Antigliadin Igm	6	13.3
Positive Antigliadin Igm	39	86.7

Table (5) Relation of serum levels of IgA, IgG, IgM antigliadins antibodies and some GIT symptoms in the studied cases group

Positive History Of GIT Symptoms / Variables	IgA Antigliadin		IgG Antigliadin		IgM Antigliadin	
	t	P	t	P	t	p
Vomiting	0.390	<0.01	0.455	<0.01	0.784	<0.01
Constipation	0.579	<0.01	0.493	<0.01	0.528	<0.01
Diarrhea	0.362	<0.05	0.368	<0.05	0.844	<0.01
Pale, foul- smelling stool	0.043	>0.05	0.061	>0.05	0.002	>0.05
Recurrent Attacks Of Abdominal Pain	0.524	<0.01	0.577	<0.01	0.584	<0.01
Food Sensitivity	0.388	<0.01	0.501	<0.01	0.459	<0.01
Intolerance Of Particular Foods	0.449	<0.01	0.455	<0.01	0.480	<0.01

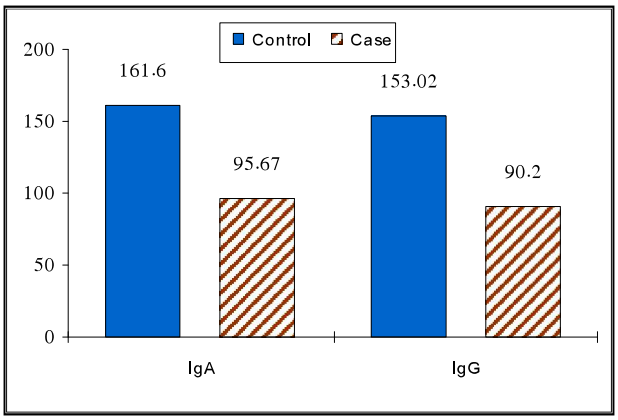

Figure (1) Behavior disorders of the studied patients.

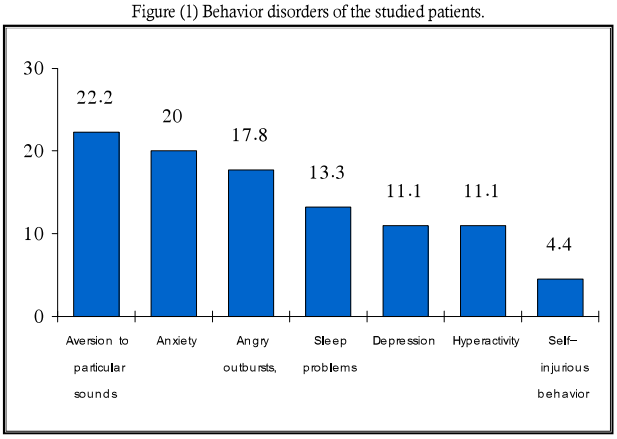

Figure (2) Comparison of serum levels of IgA- AgA, and I gG- AgA of the studied patients versus control groups.

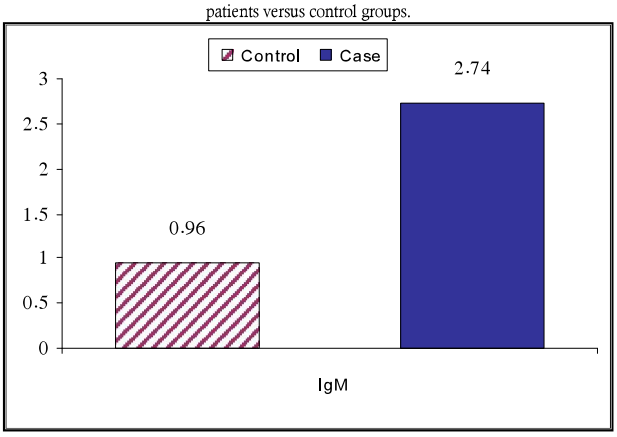

Figure (3): Comparison of serum levels of IgM-AgA of the studied patients versus control groups.