

## Possible Immunological Disorders in Autism: Concomitant Autoimmunity and Immune Tolerance

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Autism is a pervasive developmental disorder that affect children early in their life. Immunological disorders is one of several contributing factors that have been suggested to cause autism. Thirty autistic children aged 3-6 years and thirty non-autistic psychologically-free siblings were studied. Circulating IgA and IgG autoantibodies to casein and gluten dietary proteins were detected by enzyme-immunoassays (EIA). Circulating IgG antibodies to measles, mumps and rubella vaccine (M.M.R) and cytomeglovirus were investigated by EIA. Results revealed high seropositivity for autoantibodies to casein and gluten: 83.3% and 50% respectively in autistic children as compared to 10% and 6.7% positivity in the control group. Surprisingly, circulating anti-measles, anti-mumps and anti-rubella IgG were positive in only 50%, 73.3% and 53.3% respectively as compared to 100% positivity in the control group. Anti-CMV IgG was positive in 43.3% of the autistic children as compared to 7% in the control group. It is concluded that, autoimmune response to dietary proteins and deficient immune response to measles, mumps and rubella vaccine antigens might be associated with autism, as a leading cause or a resulting event. Further research is needed to confirm these findings.

Autism was only identified in 1943, and only in 1971 was it distinguished from schizophrenia (Kolvin, 1971). Soon recognized as a genetically-based biological disorder (Rutter, 1998). Autistic children are characterized by impairments in social interaction and verbal and non verbal communications and the presence of restricted and repetitive, stereotyped behaviors. The etiology of autism spectrum disorders (ASD) is largely unknown, but genetic, environmental, immunological, and neurological factors are thought to be a contributing causes. (Ashwood et al 2006). Recently, many researches has focused on neuro-immune interactions and its role in ASD because this interaction begins during embryogenesis and persist throughout an individual's lifetime.

Torres et al. (2001) and Odell et al. (2005) supported the notion of an autoimmune-based genetic make up in class II HLA-DRB04 and Class III complement C4B in the majority of autistic children. Moreover, immune

aberrations in autistic children have been hypothesized to have a link with dietary proteins particularly milk protein "casein" and wheat protein "gluten". Reichelt et al (1991), suggested that the ingested casein and gluten escaped into the circulation and act as exogenous opioids triggering the opioid receptors in the brain with the release of endorphins (opium-like effect) resulting in lack of attention and communications in those children. Furthermore, an impressive study by Vojdani et al (2003), proved that dietary peptides casein and gluten bind to CD26 and CD69 receptors on the surface of lymphocytes, inducing antibody production against these molecules. Subsequently, the same researchers (2004) reported that gluten induces the development of peptidase antibodies in autistic children. Moreover, Silva et al (2004), stated that autistic children have got a wide range of autoantibodies repertoire to a variety of their self-tissues such as Myelin Basic Protein (MBP) antibody, anti-nuclear antibody (ANA), anti-laminin

antibodies, anti-heat shock protein 60 (HSP-60), anti-caudate nucleus antibody as well as anti-casein and anti-gluten antibodies, all these overload autoimmune antibodies may be responsible for the neuro-developmental pathogenesis. Eventually, Knisberg et al (1998) and Whiteley et al (1999), reported that implementation of casein-free, gluten-free diet regimen have improved the autistic symptoms. On the other hand, several research studies suggested a neurotoxic effect of ethyl mercury containing vaccine preservative thimersol such as Measles, Mumps, Rubella vaccine (M.M.R) (Hornig et al., 2004). Moreover, Singh and Rivas 2004 have noticed dysfunctioning immune reaction to the vaccine virus strains in M.M.R with the production of abnormal antibody to 74KD molecular weight protein in autistic children. These data have put MMR vaccination in a position whether it is harmful or beneficial or has an equivocal effect in autistic children.

The present study addresses the question of immunologic disorders in autism. Autoantibodies to dietary proteins casein and gluten in autistic children, as well as, their immune response to childhood viral vaccine M.M.R antigens and CMV infection were assessed in autistic and non-autistic children.

## Materials and Methods

### Subjects

The study included 30 children (24 boys and 6 girls) with autistic spectrum disorder. Their ages ranged from 3-6 years. All were attending the outpatient clinic of Jeddah child and Adolescents Psychiatric services centre. Informed consent was taken from their parents. Patients included 22 children with autism, 6 with asperger syndrome, one with Rette syndrome and one with childhood distegrative disorder. Diagnosis was carried out according to Diagnostic and Statistical Manual of Mental Disorders (DSM IV) 1994 and ensured by the clinical psychiatric sheet. The control subjects were 30 psychiatrically-free aged 3-6 years siblings (18 boy and 12 girls). Exclusion criteria for autistic children were : physically handicapped children, organically mentally affected children,

children with no psychiatric co morbidity, children on medications.

Patients were subjected to the following:

- Full clinical Psychiatric sheet with the aim of illustration of the personal data, psychiatric symptoms, previous maternal abortion or stillbirth, developmental history, past medial and psychiatric history, and family history.
- Childhood Autism Rating Scale (C.A.R.S.) (Scopler et al., 1988) had been done for quantitative assessment of the severity of autistic criteria (mild-moderate-severe degrees) in the autistic children.
- Semi quantitative enzyme immunoassay for detection of serum IgG and IgA anti-gluten antibodies (EMMCO Diagnostics – USA). The assay is based on binding of serum autoantibodies to the gluten antigen-coated microtitre wells. After incubation and washing. Goat anti-human IgG or IgA alkaline phosphatase conjugates were added. After second incubation and washing, chromogen – substrate was added and the developed colour reaction is measured by spectrophotometer at 450 nm, 620nm. The kit contains four calibrators scores:  $\leq 0.9$  is considered normal, 1.0-2.0 has +1 score, 2.0—3.0 has +2 score and  $\geq 3.0$  has +3 score.
- Semiquantitative enzyme immunoassay for detection of circulating serum IgG and IgA against the milk protein casein (Alletess Medical Lab, USA). The assay principle and the scoring rate is the same as anti-gluten kit, previously described.
- Semiquantitative enzyme immunoassay with six calibrators, for detection of circulating serum IgG anti-measles (Date Behring – UK).
- Semiquantitative enzyme immunoassay with six calibrators, for detection of serum IgG anti-mumps (Date Behring-UK).
- Semiquantitative enzyme immunoassay-six calibrators – for detection of serum IgG anti-rubella (Dade Behring – U).
- Semiquantitative enzyme immunoassay – six calibrators – for detection of serum IgG anti – CMV (Date-Behring UK).

The above immunological procedures were carried out at the Serology department - King Fahad General Hospital (K.F.G.H) Jeddah.

## Results

Regarding symptoms of impairment in language development, 24 children (80%) demonstrated expressive language delay. 21 children (70%) developed echolalia, and 19 children (63.3%) had receptive language

delay. 17 children (50.6%) showed poor eye contact and non-verbal communication, and 20 (60.6%) showed impaired emotions and attachment (table 1).

Table 1. Symptoms of impairment in language development in autistic children.

Symptoms	Total number	Percent %
Expressive language delay	24	80
Echolalia	21	70
Receptive language delay	19	63.3
Poor Non-verbal communication	17	50.6
Impaired emotions and attachment	20	60.6

Autistic children showed increased seropositivity of IgG and IgA antibodies to dietary protein antigens, casein 83.3% and gluten 50% while in non-autistic children the seropositivity was only 10% and 6.7% respectively (table 2). Regarding the immune response to M.M.R vaccine, the autistic children showed IgG antibody response to measles, mumps and rubella viruses in only 50%, 73.3% and 53.3% respectively, meanwhile the non-autistic normal siblings

have showed 100% IgG seropositivity to the vaccinated antigens. Among the thirty autistic children, 43.3% were IgG-anti CMV seropositive versus 10% of the non-autistic ones.

Applying the Chi-square test (after transforming the percentages to  $\arcsin \sqrt{\frac{\%}{100}}$ ) we found a significant difference (Chi-square  $X^2 = 263.92$ ,  $P < 0.001$ ).

Table 2. Circulating IgG and IgA antibodies to various antigens in autistic and non-autistic children.

Antibodies	Autistic samples		Non-autistic samples		P<
	Total No. 30	Percent %	Total No. 30	Percent %	
Casein IgG, IgA	25	83.3	3	10	0.001
Gluten IgG, IgA	15	50	2	6.7	0.001
Measles IgG	15	50	30	100	0.001
Mumps IgG	22	73.3	30	100	0.001
Rubella IgG	16	53.3	30	100	0.001
Cytomegalovirus IgG	13	43.3	3	10	0.001

Table (3) showed that deficient immunological unresponsiveness to M.M.R vaccine is directly associated or linked to the degree of autism.

Table 3. Correlation between immune unresponsive autistic children to M.M.R vaccination and the severity of symptoms.

Seronegative samples	Mild to moderate autistic degree		Severe autistic degree	
	Number	%	Number	%
Measles IgG	6/15	40	9/15	60
Mumps IgG	2/8	25	6/8	75
Rubella IgG	6/14	42.8	8/14	57.2

Table (4) showed that twenty-five children were seropositive to casein antibodies, 10 children (40%) have mild to moderate degree of autism, 15 (60%) suffered from severe autistic features. Meanwhile, fifteen children were seropositive to gluten antibodies, among them 5 children (33.3%) have mild to moderate autistic features and 10 children (66.7%) have severe symptoms.

Table 4. Correlation between serum auto-antibodies (IgG and IgA) to dietary proteins casein and gluten, and the severity of autistic symptoms.

Seropositive samples	Mild to moderate autistic degree		Severe autistic degree	
	Number	%	Number	%
Casein	10/25	40	15/25	60
Gluten	5/15	33.3	10/15	66.7

## Discussion

Neuro-immunopathogenesis is a very complex, highly evolved network of interacting signals between neuropeptides and cytokines that could result in immune aberrations consistent with neuro-developmental disorders such as autism spectrum disorders (ASD) that occur in childhood.

In the present study, serum IgA and IgG anti-casein and anti-gluten were detected in a higher rate among autistic children than in normal kids (Table 2). Reichelt et al (1991), proposed the opioid theory in autism, which suggests that autism arises from early, long-term overload of the central nervous system by opioids which are most probably the exogenous incompletely-digested dietary casein and/or gluten. The theory explained that the fault may be lying in deficient barriers

in the lymphoid bowel mucosa and blood-brain barrier together with failure of intestinal and circulating peptidases to convert opioids into harmless metabolites.

In another parallel study, an informative data reported by Scifo et al. (1991), that application of very low dose Naltrexone which provides a pharmacological block of opioid action have led to improvement in the autistic hyperactivity and stereotypy.

In agreement with our findings, Lucarelli et al (1995) and Vojdani et al (2002), reported that circulating antibodies to food substances namely casein and gluten (gliadin) are detected in autistic children. Furthermore, antibodies to neural-specific antigens in the sera of those children could cross-react with dietary peptides, including milk. Moreover, Bushara (2005), reported that gluten (gliadin) intolerance per se can produce neurological disease.

Previous investigation of gut-brain interactions in children with autism, has demonstrated that more than 80% have gastrointestinal symptoms which could clarify the potential links with intestinal pathology and the effect on behavior such as in celiac disease and other inflammatory bowel diseases; and that gluten intolerance, can produce secondary neurological symptoms (Ashwood et al 2006).

In the present study, dietary exclusion of casein and gluten has been incorporated into a special diet regimen and provided to the autistic-children mothers', then after six-month follow up there were a noticeable improvement in the behavioural symptoms. A similar finding have been reported by Knivsberg et al. (1998) and Whiteley et al, (1999).

As regards M.M.R vaccination, Previous studies have reported that in 30% of autistic children, the regression symptoms occurred immediately after immunization, with detection of measles infection in ileo-caecal biopsy specimens (Wakefield et al., 1998) and another study by Montgomery et al (1999) implicated coincident mumps infection with inflammatory bowel disease and autism. These findings raised the question, whether are all autistic children vulnerable to the polyvalent M.M.R vaccine or to measles and mumps infections? In the present study, when we investigated the immunological memory of those children in response to M.M.R vaccine surprisingly, and we demonstrated failure of IgG production in most of the kids where only 50%, 73.3% and 53.3% were positive, Meanwhile all non-autistic siblings showed competent immunological response. Furthermore the immune unresponder ratios coincide with the severity of autistic degree (Table 3). Several contradictory data have been reported by many researchers that leave the MMR vaccine in an equivocal position in relation to autism (Berney, 2000).

Our findings could be a result of neuro-immunological dysfunction with imbalance in immunoglobulin production. Abnormal concentrations of plasma Ig classes have been observed in ASD children (Ashwood et al., 2003). A skewing in Ig isotype with increase in IgG2 and IgG4 has been reported by Croonenberghs et al. (2002). Elevated levels of IgG1 and IgG4 and skewed IgM concentration has also been observed by Trajkovski et al. (2004). These findings are indicative of aberrant immune response with increased susceptibility to infections and may be to vaccination as well. In contrast, Taylor et al (1999), studied 498 autistic, MMR-vaccinated cases that showed no evidence to link MMR vaccination with the incidence or regression of autism. At this stage, we can conclude that M.M.R vaccine may not be a cause of autism, but the deficient response is an outcome of the disturbed neuro-immune integrity.

To have more data on the association of aberrant immune responsiveness to infectious agents and autism, we studied immunity to CMV and demonstrated that 43.3% of the autistic children have IgG positivity to CMV. Whether this is a congenital or acquired infection and whether it plays a role in the pathogenesis of the disease, is still obscure and needs further investigation.

In conclusion, autism is considered an extreme challenge to integrative medicine, in which a cooperative team of different specialities: neurologists, psychiatrists, immunologists and genetic experts can work together to provide helpful consultation to these miserable kids.

According to our data, a challenging notice in autistic children is their deficient immune response to viral vaccine M.M.R, and on the other hand, increased autoantibodies production to dietary proteins casein and gluten. A controversial situation that needs further investigation.

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