INTRODUCTION

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Autism is a severe neurodevelopmental disorder that is characterized by impairment in verbal and non-verbal communication, imagination and reciprocal social interaction ***(Dalton et al., 2004)***. The prevalence of autism has surged in recent years ***(Kid, 2002)***. The etiology of autism is not well understood. It may occur as a result of exposure to environmental triggers in presence of genetic predisposition ***(Dalton et al., 2004)***.

A possible role of autoimmunity to brain in autism is postulated as evidenced by the presence of brain-specific autoantibodies in many autistic children ***(Vojdani et al., 2002; Mostafa and Refai, 2007; Mostafa et al., 2008a)***. Other clues for the occurrence of autoimmunity in autism include; the increase of autoimmune disorders among autistic families ***(Sweeten et al., 2003; Mostafa and Refai, 2007; Mostafa et al., 2008a; Mostafa and Kitchener, 2009)*** and the imbalance of T-helper (Th) 1/Th2 subsets toward Th2, which are responsible for allergic response and production of antibodies in some autistic children ***(Cohly and Panja, 20054)***.

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Gangliosides are a family of sialylated glycosphingolipids expressed in the outer leaflet of the plasma membrane of the cells of all vertebrates and are particularly abundant in the nervous system, in particular at synapses ***(Argia et al., 2008; Zitman et al., 2008)***. Gangliosides are thought to play important roles in memory formation, neuritogenesis, synaptic transmission, and other neural functions ***(Sugiura et al., 2008)***. Various kinds of

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gangliosides could be separated individually by using centrifugal partition chromatography ***(Kato and Hatanaka, 2008)***. The administration of exogenous gangliosides seems to improve nerve regeneration ***(Ribeiro et al., 2008)***. Gangliosides have a hydrophilic sugar chain that contains antigenic determinants and a hydrophobic ceramide. In humans, gangliosides elicit a T-cell independent IgM response ***(Ravindranath et al., 2005)***. Ganglioside M_1 (GM_1) is the most abundant ganglioside in neural membranes. It may be an autoantigen through the galactose-galactosamine part of its sugar moiety ***(Yuki, 19~~97~~98)***.

In immune-mediated neurological disorders, various autoantibodies against neuronal tissues have been discovered. Although some of these antibodies have been found to correlate with the pathomechanism of the disease, most remain disease markers with unknown pathomechanisms ***(Watanabe and Arimura, 2008)***. A correlation between specific neuropathies e.g., (autoimmune peripheral neuropathies and Gullian Barrè syndrome) and antiganglioside ~~s~~ autoantibodies has been confirmed and many neurologists attempt to lower titers of antiganglioside autoantibodies ***(Ravindranath et al., 2005; Conrad et al., 2007; Kaida et al., 2006 and Schessl et al., 2007)***.

It is not known what triggers the release of anti-ganglioside antibodies. The mechanism may involve the intestinal immune system response to ingested gliadin, a component of wheat gluten, in people with gluten sensitivity such as autistic patients ***(Sabayan et al., 2007)***. Altered patterns of gangliosides in the CNS might reflect important correlates of pathogenesis in autism ***(Nordin et al., 1998)***.

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AIM OF THE WORK

Since autism may be one of the pediatric autoimmune neuropsychiatric disorders, this study was designed to investigate the frequency of serum anti-ganglioside (~~G~~M1) antibodies (aGM_1-Abs), as index off~~or~~ autoimmunity to brain in autistic children. The relationship between these antibodies and important disease characteristics (such as a family history of autoimmunity) was also studied.

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AUTISM

Definition:

Autism is a severe neurodevelopmental disorder characterized by impaired communication, social interaction and imagination that is often accompanied by repetitive and stereotyped behavior ***(Moy et al., 2006).*** It develops before the 36th month of age and persists into adulthood causing life long disability ***(Ashwood and Van, 2001)***.

Autism is recognized now as a heterogeneous syndrome with a broad range of behavioral symptoms and severity. The spectrum of autism related disorders is collected under the umbrella of pervasive developmental disorders (PDDs) ***(American Psychiatric Association, 1994)***. All of them demonstrate characteristic deficits in communicative and social skills, varying however, in pervasiveness, severity and onset. This group comprises autistic disorder (previously referred to as classic autism). Asperger's syndrome, Rett's disorder, childhood disintegrative disorder, and pervasive developmental disorder not otherwise specified (PDD NOS) ***(Elena et al., 2002)***.

Historical Review:

In ***1943, Leo Kanner*** first described 11 cases of what he termed autistic disturbance of affective contact. In these cases, there was an inability to relate to people in usual ways. Kanner also noted unusual responses to the environment, which could include both stereotyped behavior and resistance to change or insistence on sameness, as well as unusual aspects of the child's

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~~communication skills, such as tendency to echo language (echolalia). Kanner believed that the condition was not associated with mental retardation because the children looked intelligent and did well on some parts of intelligence quotient (IQ) tests. As time went on, it became clear that most children function in the mentally retarded range. However, consistent with Kanner's original observation, it continues to be the case that marked scatter in skills with occasional "splinter skills" is often observed. Kanner mentioned that parents were unusually well educated or successful in their occupations. This led to the notion that autism might somehow result from pathological patterns of care. Considerable evidence suggests this is not the case. There is no particular bias in terms of social class distribution of autism if factors that control for case ascertainment are controlled for. It is also the case that parents of autistic children do not exhibit specific deficits in parenting or other aspects of child care and are not likely to have other psychiatric disabilities at an increased rate, with the exception of mood disorders and a range of developmental difficulties. In the early 1960s, a growing body of evidence began to accumulate to suggest that the condition resulted from a neuropathological process. A landmark in classification occurred in 1978 when Micheal Rutter proposed a definition of autism based on:~~

1. ~~Social delay and deviance (not just due to mental retardation).~~
2. ~~Communication problems (not just due to mental retardation).~~
3. ~~Unusual behavior such as stereotyped movements and mannerisms (insistence on sameness).~~
4. ~~Onset before the age of 30 months *(Volkmar et al., 1994).*~~

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Rutter's definition and the growing body of work on autism were influential in the definition of the condition of DSM-III. In DSM-III, the condition was first recognized and placed in a new class of disorders- the pervasive developmental disorders. Successive editions of the World Health Organization's "International Classification of Diseases" (ICD) and the American Psychiatric Association's "Diagnostic and Statistical Manual of the Mental Disorders" (DSM) have reflected changing ideas of autism and related disorders. Almost 50 years after Kanner's first description, the latest versions of ICD (ICD10) ***(World Health Organization, 1992)*** and DSM IV ***(American Psychiatric Association, 1994)*** provided virtually identical definitions of autism and autistic-like disorders ***(Volkmar et al., 1994)***.

In both systems, the overall construct of autistic and autistic like behavior problems has been given the name pervasive developmental disorders. The term "pervasive" was meant to emphasize that in autism, development was disturbed over a range of different domains, in contrast to the relatively more delineated difficulties of the specific developmental disorders and the centrality of cognitive problems in mental retardation. The term "developmental" implies that individuals with these conditions suffer from disturbances in the normative unfolding of multiple developmental competencies, including social relations and communication. These disorders have their onset in the first years of life, and developmental disorders have important implications throughout the life span ***(Sophie, 2002)***.

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Epidemiology of autism:

A. Prevalence of Autism:

Autism was initially felt to be a fairly rare illness (less than 5 in 10,000), but over the last twenty years there has been an explosive increase in incidence, growing on average around 25% per year in some areas ***(Jepson, 2003)***. Studies show prevalence rates ranging from 10 to 20 per 10,000 children ***(Dalton et al., 2004)***. In the United states, it is currently believed to affect 1 out of every 250 individuals on average (up to 1 in 150 in some areas) ***(Jepson, 2003).***

B. Sex Ratio:

Studies based on on both clinical and epidemiological samples have suggested a higher incidence of autism in boys than in girls with male: female ratio of 3:1 ***(Fombanne, 2003)***.

C. Social Class:

Although early studies supported Kanner's impression of an association between autism and a higher socioeconomic status, most epidemiological studies published in the 1980s and 1990s have faild to reveal such association. Autism is clearly seen in all social classes ***(Volkmar et al., 2004)***.

Etiology of autism:

In 10-30% of cases, there is an identifiable etiology (secondary autism). Some of the frequently quoted causes are herpes simplex encephalitis which involves one or both temporal lobes, intra uterine cytomegalo virus or rubella infections, intra-

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~~uterine exposure to thalidomide or valproate, chromosomal anomalies like fragile X or Angelman syndromes, genetic disorders such as inadequately treated phenyl ketonuria (PKU), tuberous sclerosis or Cornelia de Lange syndrome, and many others ***(Gillberg and Colman,1996)***. In the majority of cases, there is no obvious cause (primary autism) ***(Cohen and Volkmar, 1997)***.~~

~~The causes of autism are multifactorial ***(Behrman, 2004)***. Autism is caused when a child with an appropriate genetic susceptibility is exposed to a number of environmental insults resulting in a complex series of interactions in several body systems, primarily the central nervous system (brain), the gastrointestinal system (the gut) and the immunological system (body defense) (Fig. 1). Each child with autism is a unique individual and has unique biochemistry that has some how become disordered ***(Jepson, 2003)***. Causes of autism include:~~

~~1-Genetic causes:~~

~~The neuroanatomic findings in monozygotic twin pairs with autism support the role of genetic liability in autism ***(Kates et al., 2004)***. The recurrence risk for autism after the birth of an autistic child is 60 to 150 times more than the population base rate. Epidemiologically based, same gender twin studies have reported higher concordance rates for autism among identical twins than among non identical twins. The mode of genetic transmission is unclear. The marked fall off in rates of autism that occurs from identical to non identical twins or siblings suggests that a small number of interacting genes rather than~~

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~~one single gene is involved, with estimates of genes involved ranging from 2 to 20. ***(Cook, 2001)***.~~

~~The genes of autism have been found to affect different steps of cortical development, including proliferation of neuronal progenitor cells, neuronal migration and maintaining integrity of the pial surface ***(Mochida and Walsh, 2004)***. Several full genome searches for susceptibility loci in autism using affected sibling pairs have been performed. Although several areas of the genome (i.e, on chromosome 7q, 1, 2, 6, 13 and 16) have been identified as regions of interest, currently no specific variation in a specific gene has been firmly established as a susceptibility gene for autism ***(Cook, 2001)***. The most promising may be the findings of deletions and duplications in chromosome 15 affecting the transport of the neurotransmitter serotonin ***(Cook et al., 1997)*** but many other chromosomal loci are also being considered ***(Gillberg, 1998)*** such as HLA genes and their products ***(Lee et al., 2006)***.~~

~~*Metallothionein (MT) dysfunction:*~~

~~MT is a family of proteins that controls the copper/zinc ratio in the body. MT dysfunction in autism was described by William Walsh, who took extensive biochemical analyses of over 500 autistic patients, and discovered that almost universally, these children have abnormal copper/zinc ratio with high body copper and low body zinc. Other functions of MT in the~~

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body include development of brain neurons, detoxification of heavy metals, maturation of the gastrointestinal tract (GIT), anti-oxidation, boosting immune function and delivery of zinc to cells. Because MT synthesis is enhanced by estrogen and progesterone, it would explain the male sex predominance (4: 1) seen in autism. MT dysfunction could be caused by a genetic defect, or an environmental insult that disables MT ***(Jepson, 2003)***.

2-Mitochondrial causes:

A likely etiological possibility in autism may involve a mitochondrial dysfunction with concomitant defects in neural oxidative phosphorylation within the central nervous system ***(Lombard, 1998).*** Autism may be a disorder of fatty acid metabolism due to a possible dysfunction of mitochondrial long chain acyl CoA dehydrogenase enzyme responsible for the beta oxidation of unsaturated fatty acids in the mitochondria ***(Clark-Taylor, 2004).*** This hypothesis is supported by a frequent association of lactic acidosis and carnitine deficiency in autistic patients. Also because the mitochondria are vulnerable to wide array of endogenous and exogenous factors which appear to be linked by excessive nitric acid production ***(Lombard, 1998).***

In ***2005, Mostafa and coworkers*** reported that brain energy metabolism of many autistic children is low due to summation of several factors including low plasma polyunsaturated fatty acids (PUFAs) and/or disturbed

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~~mitochondrial function as evidenced by decreased serum carnitine and increased plasma lactate levels.~~

~~There are autistic children who come from families with patterns of maternal inheritance of psychiatric disabilities. This opens up the possibility of errors of mitochondrial DNA **(Gillberg, 2000).** Mitochondrial DNA (mtDNA) is strictly maternally inherited and does not recombine.. Mitochondrial DNA mutates more than 10 times more rapidly than nuclear DNA. It has a greater exposure to oxidative stress because it is exposed to oxygen free radicals generated by oxidative phosphorylation and there is an absence of a protective histone coat. In addition, mtDNA has no effective repair system for DNA damage **(Gillberg and Coleman, 2000).** In Rett syndrome, a pathologic role of mitochondria has also been proposed based on ultrastructural abnormality in mitochondrial number and size in skin and muscle biopsies **(Singer and Naidu, 2001)**. Strategies to augment mitochondrial function either by decreasing production of endogenous toxic metabolites, reducing nitric oxide production, or stimulating mitochondrial enzymes activity may be beneficial in treatment of autism **(Lombard, 1998)**.~~

~~3 Neurobiological Causes~~

~~The three principal neurotransmitter types in the brain are:~~

1. ~~The monoamine neurotransmitters, which are the catecholamines (dopamine, norepinephrine, and epinephrine), serotonin, acetylcholine and histamine.~~

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2- The amino acid neurotransmitters, which include the inhibitory actions of γ-aminobutyric acid (GABA) and the excitatory glutamate.

3- The neuropeptide neurotransmitters as corticotropin-releasing factor, somatostatin release-inhibiting factor, neurotensin and cholecystokinin ***(Kaplan, 2000)***.

Synaptic transmission of multiple neurotransmitters needs the neurobiological effect of Acetyl L-Carnitine ***(Traina et al., 2004).***

(A) Serotonin and autism:

Serotonin is formed by hydroxylation and decarboxylation of tryptophan ***(Narita et al., 2002).*** Recent studies have provided that plasma docosahexaenoic acid (DHA) which is a polyunsaturated fatty acid (PUFA) is also involved in dopamine and serotonin metabolism ***(Innis, 2000).***

Serotonin is a neurotransmitter in the central nervous system and it is involved in early neurogenesis ***(Whitaker - Azmilia, 2001).*** Serotonin is known to play a role in brain development and it regulates both the development of serotonergic neurons and target tissues ***(Narita et al., 2002).*** Disorders of serotonin metabolism ***(Delong, 1999)*** or disruption of serotonergic development can leave permanent alterations in brain function and behavior and this may be the case in autism ***(Whitaker -Azmitia, 2001; Narita et al., 2002)***. Serotonin depletion early in life might be a factor related to the increased

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~~neuronal cell numbers and/or macrocephaly found in some autistic brains *(Gillberg and Coleman, 2000)*.~~

~~***Mostafa et al. (2003 and 2008b)*** reported elevated levels of serum serotonin in 70% and 55%, respectively of autistic children and its levels correlated positively with disease severity and manifestations. However, levels of 5-hydroxyindoleacetic acid (the end product of serotonin metabolism) have not been found to be elevated in most studies of the cerebrospinal fluid in children with autism *(McDougle, 2002).*~~

~~Elevated serum serotonin levels do not necessarily mean that this translates to high levels in the central nervous system A direct measurement was made at autopsy of a child with leukodystrophy who had elevated serotonin levels recorded in his whole blood during his life time, the brain level of serotonin was diminished compared to a control who died at the same day *(Gillberg and Coleman, 2000)*. In favor of the likelihood of low serotonin level in the brain in some patients with autism, is the observation that non-selective serotonin reuptake inhibitor drugs (SRIs) as clomipramine and selective serotonin reuptake inhibitors (SSRIs) as fluroxamine which increase the amount of serotonin at the receptor site, have improved specific symptoms in up to half of the patients with autism *(McDougle, 2002)*. Results from a positron emission tomography (PET) brain imaging study showed that changes in brain serotonin synthesis capacity are disrupted in autistic children *(Mc Dougle, 2002)*.~~

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~~One indirect measure of the success of a new treatment in autism is the improvement of serotonin levels in the blood of autistic patients i.e. return to normal in those who start treatment with abnormal levels, whether high or low **(Gillberg and Coleman, 2000)**.~~

~~*(B) Catecholamines and autism:*~~

~~***Emst et al. (1997)***, demonstrated low prefrontal dopaminergic activity in autistic children compared to controls measured by PET. In addition, children with PKU clinically diagnosed as having autism, have low brain dopaminergic activity **(Gillberg and Coleman, 2000)**.~~

~~*(C) Cholinergic activity and autism:*~~

~~Abnormality of Cholinergic activity is reported in the cerebral cortex and basal forebrain in autism. So, the intervention in autism should be based on cholinergic receptor modulation **(Perry et al., 2001).**~~

~~*(D) Nicotinic receptors and autism:*~~

~~Nicotinic receptor abnormalities in the cerebellar cortex, and purkinje cell loss, were observed in multiple lobules throughout the vermis and hemispheres in autism. These findings indicate a loss of the cerebellar nicotinic 4 receptor subunit in autism which may relate to the loss of Purkinje cells and a compensatory increase in the 7 subunit. It remains to be determined how these receptor abnormalities are involved in neurodevelopment in autism. Since nicotinic receptor agonists~~

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enhance attentional function and also induce an elevation in the high affinity receptor, nicotinic therapy in autism may be worth considering ***(Lee et al., 2002).***

(E) Glutamate neurotransmitter and autism:

N-methyl-D-aspartate (NMDA) subtype of glutamate receptor, which is central to the developmental processes including neuronal migration, differentiation, and plasticity, is receiving an increasing attention in its relation to autism. In one study, no difference was found between NMDA receptor antagonist (amantadine) and a placebo on parent ratings, although clinician-rated measures of hyperactivity and inappropriate speech showed significant improvement ***(McDougle, 2002).*** Of interest, one mechanism of action underlying the relative efficacy of atypical antipsychotics, such as risperidone, for PDDs may be the suppression of glutamate release ***(Lavenstein, 2003).***

4- Biomedical Causes:

(A) Low fatty acids:

Changes in the metabolism of fatty acids are evidenced in several psychiatric disorders (e.g. depletion of omega-3 fatty acids in bipolar disorder and major depression) ***(Jepson, 2003)***. In one study, a marked reduction in the level of docosahexaenoic acid (DHA) was reported in 23% of autistic subjects ***(Vancussel, 2001)***.

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