INTRODUCTION

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).* There are no defined mechanisms of pathogenesis or curative therapy presently available for autism *(Theoharides et al., 2008).* It is thought that autism could result from an interaction between genetic and environmental factors with oxidative stress as a potential mechanism linking the two. One genetic factor may be altered oxidative-reductive capacity in autism *(Ming et al., 2005).* Oxidative stress has been implicated in the pathogenesis of many major psychiatric disorders, as the brain has comparatively greater vulnerability to oxidative damage *(Ng et al., 2008).*

Oxidative damage mediated by reactive oxygen species (ROS) results in the generation of deleterious by-products. ROS attack the polyunsaturated fatty acids constitutive of cellular membranes resulting in formation of end products of lipid peroxidation (LPO) *(Basu, 2008).* Isoprostanes are prostaglandin-like bioactive compounds that are biosynthesized in vivo independent of cyclooxygenases, principally through free-radical catalyzation of arachidonic acid. Isoprostanes are now considered to be reliable biomarkers of oxidative stress and LPO *(Basu, 2008).*

Glutathione, the most abundant low molecular weight thiol compound synthesized in cells, plays an important role in anti-oxidant defense and detoxification reactions. It is primarily synthesized in the liver by the transsulfuration pathway. Glutathione peroxidases catalyze the reduction of H_2O_2 or organic hydroperoxides to water or corresponding alcohols using reduced glutathione *(Margis et al., 2008)*. Deficits in glutathione have been implicated in aging and many of diseases including Alzheimer's disease, Parkinson's disease, cardiovascular disease, cancer, Down syndrome and autism *(Forman et al., 2008; Reed et al., 2008)*. Studies suggest a role for vitamin C and E in reducing LPO *(Block et al., 2008)*.

Autistic children exhibit evidence of oxidative stress and impaired methylation, which may reflect effects of toxic exposure on sulfur metabolism. A "redox/methylation hypothesis of autism" is described, in which oxidative stress, initiated by environment factors in genetically vulnerable individuals, leads to impaired methylation and neurological deficits secondary to reductions in the capacity for synchronizing neural networks *(Deth et al., 2008)*. Understanding the role of oxidative stress may illuminate the pathophysiology of autism, its environmental and genetic influences, new treatments, and prevention *(McGinnis, 2004)*. Recently, hyperbaric oxygen therapy has increased in popularity as a treatment for autism *(Rossignol et al., 2007)*.

AIM OF THE STUDY

This study was designed to assess oxidative stress in a group of Egyptian autistic children through measurements of plasma F2-isoprostane (as a marker of lipid peroxidation) and plasma glutathione peroxidase (as an antioxidant marker). The relationship between oxidative stress and important disease characteristics including; disease severity, allergic manifestations and a family history of autoimmunity was also studied.

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) *(Korvatska et al., 2002)*.

Epidemiology of autism:

A. Prevalence of Autism:

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 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., 2005)*.

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-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 *(Dalton et al., 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) (figure 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 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., 2005)*.

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 body include development of brain neurons, detoxification of heavy metals, maturation of the gastrointestinal tract (GI), 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- Environmental insults:

A) *Vaccines:*

Fish in our diet and dental amalgams in our mouths are common sources of mercury, but by far the largest exposure to our infants are from vaccinations. Thimerosal is a preservative

that is included in many vaccines to prevent bacterial contamination and thus, prolonging shelf life and facilitating multiuse vials. It consists of approximately 50% ethyl mercury. As noted above, mercury can be highly toxic, even in small doses. Some infants receive up to 100 times the Environmental Protection Agency (EPA) recommended safe level of mercury based on adult weights, in one day. We immunizie newborns with hepatitis B vaccines as early as the day of birth. In fact, infants' immune systems and neurological systems are too immature to handle such a toxic load. However, most children seem to be able to tolerate it just fine, but we believe that many children, who are genetically predisposed, are being adversely affected. Several databases have reported an alarming increase in the incidence of autism in the last 20-year period (over 1000% increase was reported in California). It is also interesting to note that in the same period, the number of immunizations that a child receives before the age of two has increased from 8 in 1980 up to 33 in the year 2001, and more are being developed. When hepatitis B and Hemophilus influenza B (HIB) vaccines were added to the schedule in the early 1990s the load of mercury to our children was doubled. The FDA has asked that vaccine manufacturers to remove thiomerosal from their vaccines, but fell short of demanding a recall of the existing thiomerosal-containing product. It is interesting that thimerosal was taken out of animal vaccines in USA a decade ago because it was felt to be unsafe. There is

ample evidence that manufacturers of vaccines knew about the dangers of thimerosal as early as 30 years ago *(Jepson, 2003).*

In a study conducted by *Parker (2004),* there was no link between thimerosol containing vaccines and autistic spectrum disorders. However, other studies have shown that there is a direct relationship between increasing doses of mercury from thimerosol-containing vaccines and neurodevelopmental disorders. They recommended that thimerosol should be removed from all vaccines. Although hypothetically there is a vulnerability to MMR vaccine to induce autism in 10 percent of the children with autism, several studies provided strong evidence against the hypothesis that MMR vaccination causes autism *(Madsen et al., 2002)*. Any how, There is a not enough evidence currently to prove the association between vaccines and autism *(Jepson, 2003).*

In a recent Egyptian study, autistic children, had significantly higher levels of blood mercury than healthy controls. In addition, patients with severe autism had significantly higher blood mercury levels than patients with mild to moderate disease *(Mostafa and Refai, 2007)*.

B) Seasons of birth:

Children with autism have a greater incidence of birth in March and in August. Such variations might point to seasonal factors such as infections, weather, or diet. It would seem that if

there are seasonal influences on the development of autism, these are fairly weak and inconsistent *(Stevens et al., 2000)*.

3. Immunological abnormalities:

Reports of findings of many systemic immune abnormalities in autistic patients over the past 30 years have led to speculation that autism may represent, in some patients, an immune mediated or autoimmune disorder *(Ashwood and Dewater, 2004)*. Abnormalities of both humoral and cellular immune functions have been described in some studies of children with autism and include decreased production of immunoglobulins or B and T-cell dysfunction *(Cohn et al., 2005)*. Early studies suggested that prenatal viral infections might damage the immature immune system and induce viral tolerance *(Stubbs and Crawford, 1977)*, while later studies showed altered T-cell subsets and activation, consistent with the possibility of an autoimmune pathogenesis *(Gupta et al., 1998)*. *Odell et al. (2005)* reported a four-fold increase in the serum complement 4B (C4B) null allele (i.e., no protein produced) in children with autism compared to controls.

Studies of peripheral blood of autistic children have shown a range of abnormalities, including T-cell, B-cell and NK-cell dysfunction, autoantibody production increased pro-inflammatory cytokines *(Singh et al., 1997; Gupta et al., 1998; Jyonouchi et al., 2001; Singh, 2002; Vojdani et al., 2002)*. Shifts observed from Th 1 to Th2 lymphocytes subsets *(Jepson, 2003)*. Associations with HLA-DR4 have suggested the possibility that

autoimmunity against brain antigens may contribute to the neuropathology of autism *(Van Gent et al., 1997; Torres et al., 2002)*. Decreased immunoglobulin subsets and an effect of maternal antibodies have also been proposed as pathogenic factors *(Dalton et al., 2003)*. Since systemic immune findings in autism have not been followed in the same patients over time, it is not clear whether they reflect true immune dysfunction or may represent dysmaturation that changes with age *(Zimmerman, 2005)*.

Autoimmunity is an abnormal immune reaction in which the immune system becomes primed to react against body organs, and the end result is autoimmune disease. Several factors contribute to the pathogenic mechanism of autoimmune diseases. These illnesses are commonly believed to be triggered by infectious agents *(Bach, 2005)*. Further, they are generally linked to genes that control immune responses. Abnormalities of T lymphocytes and the production of autoantibodies are features in these diseases *(Levin et al., 2004)* and they involve hormonal factors. *(Arnson et al., 2007; Orbach and Shoenfeld, 2007)*. Some clues suggesting the pathogenetic role for autoimmunity in autism include:

- Autism displays increased frequency of genetic factors linked to some autoimmune diseases for e.g., HLA and C4B null allele *(Warren et al., 1992; Daniels et al., 1995; Torres et al., 2002).*

- In a very recent study, in *2009*, *Li and coworkers* found increased levels of proinflammatory cytokines (tumour

necrosis factor -alpha, IL-6 and granulocyte macrophage-colony stimulating factor), Th1 cytokine (interferon-gamma) and chemokine (IL-8) in the brains of autistic patients compared with the controls.

- Autism often occurs in conjunction with a family history of autoimmune diseases, e.g., multiple sclerosis, rheumatoid arthritis, etc. *(Comi et al., 1999; Sweeten et al.2003; Padro et al., 2005; Mostafa and Refai, 2007; Mostafa et al., 2008a; Mostafa and Kitchener, 2009)*. However, regarding which particular autoimmune disorders are increased, *Comi and associates (1999)* and *Mostafa et al. (2008a)* found a higher familial rate of rheumatoid arthritis, whereas *Sweeten and associates (2003),* found an increased occurrence of Hashimoto's thyroiditis and rheumatic fever. The increased frequency of rheumatic fever in the families of autistic probands could be related to the increased expression of the B-cell antigen D8/17 previously reported in autistic subjects *(Hollander et al., 1999)*. Increased expression of D8/17 was originally found in patients with rheumatic fever *(Zabriskie et al., 1985)* and subsequently in patients with obsessive-compulsive disorder and tic disorders in which an autoimmune pathology is suspected *(Swedo et al., 1997)*. Concerns about the reliability of the D8/17 assay, however, have been raised *(Hamilton et al., 2003)*.

- Asthma and allergies were often reported in mothers of children *(Croen et al., 2005)*. Possible mechanisms to be considered for explanation of this association include a

shared genetic susciptibility to both immunologic diseases and autism *(Warren et al., 1996; Ginn et al., 1998)*. Passive transfer of antibodies to neural tissue or a more likely scenario which is direct impact on fetal brain development via altered levels of circulating cytokines *(Mehler and Kessler, 1998)*. There is a strong evidence that the inflammatory cytokine IL-6 can cross the placenta *(Zaretsky et al., 2004)*, and dysregulation of IL-6 has been implicated in the pathology of nervous system disturbances *(Jarskog et al., 1997)*. It is also possible that the immune response of the child, rather than that of the mother, may be the primary abnormality in autism, leading secondarily to brain dysregulation.

- Like other autoimmune diseases, autism also involves hormonal factors, e.g., secretin, oxytocin, etc. *(Yang et al., 2004; Koves et al., 2004)*. Autism and autoimmune diseases also show an association with enviromental risk factors *(Tchorzewski et al., 2000)* such as infectious agents, in particular viruses *(Korvatska et al., 2002)*.

- A new form of inflammatory bowel disease (ileocolonic lymphonodular hyperplesia) was reported in a cohort of autistic children *(Uhlmann et al., 2002)*.

- Some autistic children showed some response to immunotherapy *(Gupta, 2000)*.

- Circulating auto-antibodies directed against CNS antigens have been described in patients with autism, reacting to

myelin basic protein (MBP) *(Singh et al., 1998; Silva et al., 2004)*, frontal cortex *(Todd et al., 1988)*, cerebral endothelial cells *(Connolly et al., 1999)* and neurofilament proteins *(Singh et al., 1997)*. One study was conducted by *Vojdani et al. (2002)*, who measured autoantibodies against nine different neuron-specific antigens and three cross-reactive petides in the sera of autistic subjects. The antigens were: MBP, myelin associated glycoprotein, ganglioside, sulfatide, chondroitin sulfate, myelin oligodendrocyte glycoprotein, α-β-crystallin, neurofilament proteins, and tubulin. The three cross-reactive encephalitogenic protein peptides include; Chlamydia pneumoniae, streptococcal M protein and milk butyrophilin. Autistic children showed the highest levels of IgG, IgM and IgA antibodies against all neurologic antigens, as well as the three cross-reactive peptides. The presence of auto-antibodies in serum from autistic patients might imply that autism is an autoimmune disorder *(Pardo et al., 2005)*. Recently, *Mostafa and Refai (2007)* and *Mostafa et al. (2008a)* reported seropositivity for antineuronal and antibodies in 67.5% and 62.5%, respectively of Egyptian autistic children.

4-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 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, 1998).* 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