

## Original article

# Antineuronal antibodies in autistic children: relation to blood mercury

**Background:** It was recently suggested that autism, a severe neurodevelopmental disorder, may involve an autoimmune pathogenesis. Mercury (Hg) is a potential risk factor for autoimmunity in autistic children.

**Objective:** We sought to investigate the expression of antineuronal antibodies, as an index of autoimmunity to brain, in autistic children. The potential relationship between blood mercury and these antibodies was also investigated.

**Methods:** Forty autistic children (20 with mild to moderate and 20 with severe disease) were studied in comparison to 40 healthy children. After complete clinical and neuropsychiatric evaluation, serum antineuronal antibodies and blood Hg levels were estimated.

**Results:** Autistic children had significantly higher seropositivity for antineuronal antibodies (67.5%) than healthy controls (5%). Similarly, the former group had significantly higher blood Hg levels than the latter ( $p < 0.0001$ ). Seropositivity of antineuronal antibodies had a significant positive association with elevated blood Hg, which was found in 70% of autistic children, ( $p < 0.0001$ ). In addition, the two markers were positively associated with some parameters such as the family history of autoimmunity, autistic severity and some important clinical manifestations of autism (mental retardation, behavioral abnormalities and autistic regression) as well as EEG abnormalities.

**Conclusion:** Autism may be, in part, one of the pediatric autoimmune neuropsychiatric disorders. Such autoimmunity may be triggered by environmental Hg exposure. Further studies are warranted to enforce these concepts. If these assumptions could be proved, routine assessment of serum antineuronal antibodies and blood mercury in autistic children would be mandatory. Studies assessing the role of immunotherapy and Hg chelators as new therapeutic modalities for autism are also recommended.

**Keywords:** Antineuronal antibodies; autism; autoimmunity; children; heavy metals; EEG; mercury.

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## INTRODUCTION

Autism is a severe neurodevelopmental disorder characterized by impaired communication, social interaction and imagination that is often accompanied by repetitive and stereotyped behavior<sup>1</sup>. It develops before the 36 month of age and persists into adulthood causing life long disability<sup>2</sup>. The prevalence of autism has surged in recent years<sup>3</sup>. The etiology and pathogenesis of autism is not well understood<sup>4</sup>. In view of the possible multifactorial cause, autism can occur as a result of environmental neurotoxicant mercury (Hg) exposure in presence of genetic predisposition<sup>5</sup>.

Several sources of toxic Hg exposure in children have been reported in literature: (1) ethyl mercury, which has been the subject of recent scientific inquiry in relation to the controversial

pediatric vaccine preservative thimerosal; (2) methyl mercury, is most commonly the result of consumption of contaminated food, particularly fish; (3) inorganic Hg, through the use of topical Hg-based skin creams and in infant teething powders; (4) metallic Hg in dental amalgams, which release Hg vapors<sup>6</sup>. In 2006, Palmer and associates<sup>7</sup> reported that for each 1.000 lb of environmentally released Hg, there was a 61% increase in the rate of autism. Thus, a logic step is to identify the source of Hg exposure in the child population and consider prevention and control of environmental pollution<sup>5</sup>. Recently, thimerosal was removed from the vaccines in USA, but it is still present in developing countries<sup>8</sup>. However, the issue of the risk of vaccination remains a philosophical one, since to date, the advantage of this policy have not been refuted, while the risk for autoimmune disease has

not been irrevocably proved<sup>9</sup>. Although Hg has been proven to be a neurotoxicant, there is lack of data to evaluate the causal relationship between Hg and autism<sup>5</sup>.

Recently, it was suggested that autoimmunity to central nervous system (CNS), evidenced by the presence of brain-specific autoantibodies, in many autistic children, may play a causal role in autism<sup>10</sup>. Mercury is one of the main candidate environmental triggers for autoimmunity in autism<sup>10,11</sup> as it binds to lymphocyte receptors and/or tissue enzymes resulting in autoimmune reaction<sup>12</sup>. Other clues for the etiopathogenic role of autoimmunity in autism, other than the presence of brain-specific antibodies include; increase of autoimmune disorders among autistic families<sup>13</sup>. Also, there is a strong association of autism with the major histocompatibility complex (MHC) for the null allele of C4B in class III region. This results in low production of C4B protein leading to repeated infections which play an important role in the development of autoimmunity. In addition, there is an imbalance of Th1/Th2 subsets toward Th2 in some autistic children<sup>10</sup>. Furthermore, there is a new form of inflammatory bowel disease (ileocolonic lymphonodular hyperplasia or autistic enterocolitis) in a cohort of autistic children leading researchers to suspect a gut-brain connection in autism<sup>14</sup>.

Immunotherapy should be initiated in autistic children when a clue of autoimmunity is evidenced by the presence of autoantibodies to CNS. Adrenocorticotrophic hormone (ACTH) prescribed in the first months of the disease cured one autistic patient. In another patient, who received ACTH 6 years after the onset of the disease, partial but definitive improvement occurred<sup>15</sup>.

Antineuronal antibodies bind to the surface membranes of neurons causing direct cytotoxic neuronal injury with subsequent impairment of neuronal function and production of diffuse brain abnormalities. These antibodies have been suggested to play a central role in the pathogenesis of neuropsychiatric systemic lupus erythematosus (NPSLE)<sup>16</sup> and pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection "PANDAS" ( e.g. Tourette syndrome and early-onset obsessive-compulsive disorder "OCD")<sup>17</sup>.

Since autism may be one of the pediatric autoimmune neuropsychiatric disorders, this study was conducted to investigate the expression of antineuronal antibodies, as an index of autoimmunity to brain, in autistic children. We also sought to investigate the relationship between the

antineuronal antibody expression and blood mercury level in a trial to pave the way for new therapeutic proposals.

## **METHODS**

### ***Study population***

This case-control study was conducted on 40 autistic children recruited from the Outpatients and Psychiatric Pediatric Clinics, Faculty of Medicine, Ain Shams University, over a period of 18 months from the beginning of September 2005 to the end of February 2007. Patients were fulfilling the criteria for the diagnosis of autism according to the DSM IV diagnostic criteria for research<sup>18</sup>. They were 31 males and 9 females. Their ages ranged between 3 and 8 years (mean  $\pm$  SD = 5.38 $\pm$ 1.85 years). Patients were classified into two groups according to the disease severity assessed by childhood Autism Rating Scale (CARS)<sup>19</sup>. Group I included 20 patients (all were males) with mild to moderate autism, their ages ranged between 3 and 8 years (mean $\pm$ SD = 5.15 $\pm$ 1.8 years). Group II included 20 patients (11 males and 9 females) with severe autism, their ages ranged between 3 and 8 years (mean  $\pm$ SD= 5.6  $\pm$  1.9 years).

Autistic children were studied in comparison to 40 age- and sex- matched apparently healthy children (31 males and 9 females) who had no clinical findings suggesting neuropsychiatric disorders, serving as controls. Their ages ranged between 3 and 8 years (mean  $\pm$ SD = 5.25 $\pm$ 1.8 years).

Healthy and autistic children who did not complete their full vaccination schedule were excluded from the study to ensure uniform environmental exposure to ethyl mercury (thimerosal). In addition, autistic patients were not enrolled in our study if they had associated neurological diseases as cerebral palsy and tuberous sclerosis. An informed written consent of participation in the study was signed by the parents or the legal guardians of the studied subjects.

### ***Study measurements:***

#### ***Clinical evaluation***

Clinical evaluation of the patients was done based on clinical history taking from caregivers, clinical examination and neuropsychiatric assessment. In addition, disease severity was assessed using CARS which rates the child on a scale from one to four in each of fifteen areas (relating to people; emotional response; imitation; body use; object use; listening response; fear or nervousness; verbal communication; non-verbal communication; activity level; level and consistency of intellectual

response; adaptation to change; visual response; taste, smell and touch response and general impressions). According to the scale, children who have scored 30-36 have mild to moderate autism, while those with scores ranged between 37 and 60 points have a severe degree of autism. Special emphasis was done on family history of autoimmune diseases, rheumatic complains (such as myalgia, arthralgia, recurrent rashes, photosensitivity and alopecia), developmental history to identify patients with autistic regression (i.e., regression in language and other skills after a period of normal development), vaccination history and full neurological evaluation (searching for history of convulsions, signs of cerebellar lesion and soft neurological signs as dysidiokinesia, asteriognosis and mixed hand preference which may be found in some autistic patients). In addition to assessment of mental age (using Stanford Binet test<sup>20</sup> to calculate the intelligence quotient "IQ") and regional brain electrical activity (electroencephalography "EEG") of autistic children, serum antineuronal antibodies and blood mercury levels of all studied children were measured.

#### *Blood sampling*

Two ml of venous blood were collected and transferred into a dry clean tube and left to clot at room temperature. Then, centrifugation was done at 3000 rpm for 5 minutes. Prompt separation of serum was done and stored at -20°C until assay of antineuronal antibodies. Another one ml of blood was collected in a heparinized vacutainer for immediate assay of blood mercury.

#### *Assessment of serum antineuronal antibodies:*

This assay employs the indirect immunofluorescence technique (EUROIMMUN Laboretorium Fur Experimentelle Immunologie, Germany). Frozen sections of primate cerebellum covering the reaction area of a biochip slide are incubated with a diluted serum sample. If the sample is positive, specific antibodies of classes IgA, IgG and IgM attach to the neuronal antigens. In a second step the attached antibodies are stained with fluorescence labeled antihuman antibodies and made visible with the fluorescence microscope<sup>21</sup>.

#### *Assessment of blood mercury*

This was done by flameless atomic absorption spectrophotometer 460, at Community Medicine Department, Ain Shams University. Since data distribution was non-parametric, autistic patients were considered to have elevated blood mercury if

their levels were above the chosen highest cut-off value (the 95<sup>th</sup> percentile of the control values which was 6 µg/dl).

#### **Statistical analysis:**

The results were analyzed by commercially available software package (StatView, Abacus concepts, Inc., Berkley, CA, USA). The data were presented as mean and standard deviation (SD) in addition to median and interquartile range (IQR) which is the difference between the 75<sup>th</sup> and 25<sup>th</sup> percentiles. Mann Whitney test was used for comparison between two groups as data distribution was non-parametric. Spearman's correlation coefficient "r" was used to determine the relationship between different numerical variables. Chi-square test was used for comparison between qualitative variables of the studied groups. For all tests, a probability (p) of less than 0.05 was considered significant.

## **RESULTS**

### **Seropositivity of antineuronal antibodies in healthy and autistic children and its relation to disease severity:**

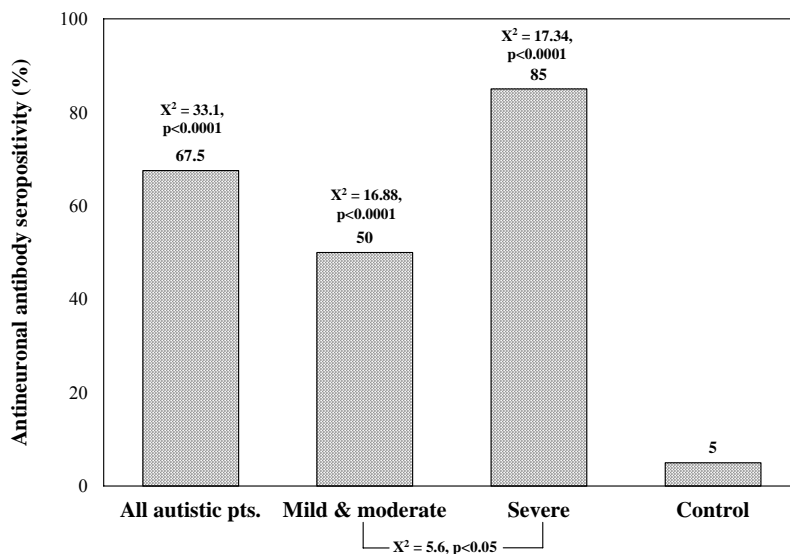
Twenty seven out of the studied 40 autistic children (67.5%) were seropositive for antineuronal antibodies (10 had mild to moderate autism and 17 had severe disease). In contrast, 5% only of healthy children were seropositive for antineuronal antibodies.

Autistic children, whether compiled in one group or subdivided according to disease severity into mild to moderate and severe, had significantly higher percent seropositivity of antineuronal antibodies than healthy controls. In addition, patients with severe autism had significantly higher percent seropositivity of antineuronal antibodies (85%) than patients with mild to moderate disease (50%). (Figure 1).

### **Blood mercury in healthy and autistic children and its relation to disease severity:**

Twenty-eight out of the studied 40 autistic children (70%) had elevated blood mercury levels above the chosen highest cut-off value (11 had mild to moderate autism and 17 had severe disease).

Autistic children, whether studied together or subgrouped according to disease severity, 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 (table 1).



**Figure 1.** Comparison between the studied groups in seropositivity to antineuronal antibodies.

**Table 1.** Comparison between the studied groups in blood mercury levels.

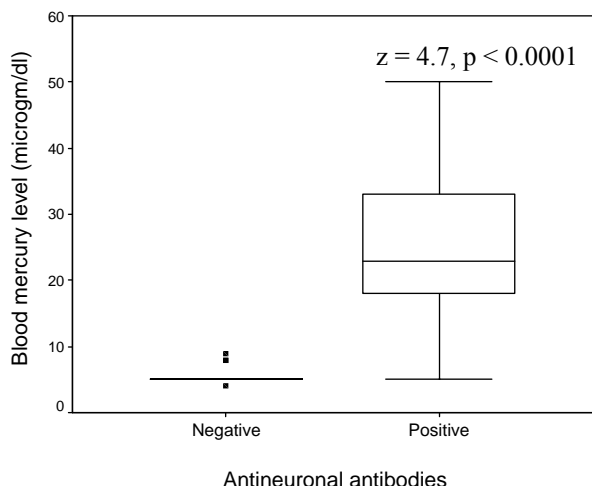
	Blood mercury (ug/dl)	
	Mean±SD	Median (IQR)
Healthy controls (n =40)	3.9±1.8	4 (3.5)
All autistic children (n = 40)	19.8±13.9	18 (26.5)
Patients with mild to moderate autism (n=20)	11.65±7.26	8 (13)
Patients with severe autism (n = 20)	28±14.3	31 (20.7)
z1 (p)	6.1 (< 0.0001)**	
z2 (p)	4.26 (< 0.0001)**	
z3 (p)	5.66 (< 0.0001)**	
z4 (p)	3.7 (< 0.0001)**	

z1: comparison between all autistic patients and controls, z2: comparison between patients with mild to moderate autism and controls, z3: comparison between patients with severe autism and controls, z4: comparison between patients with mild to moderate and those with severe autism,  $p < 0.0001$ \*\* : highly significant.

**Relationship between seropositivity of antineuronal antibodies and blood mercury levels:**

Autistic children who were seropositive for antineuronal antibodies had significantly higher blood mercury levels [mean±SD = 26.6±11.9, median (IQR) = 23 (16) µg/dl] than patients who were seronegative for these antibodies [mean±SD= 5.7±1.55, median (IQR) = 5 (1.5) µg/dl] (Figure 2). In addition, the former group had significantly higher percent of elevated blood mercury (25/27:92.6%) than the latter group (3/13:23%). Thus, there was a significant positive association between seropositivity of antineuronal antibodies and elevated blood mercury (Figure 3).

Twenty-five out of the studied 40 autistic children (62.5%) were seropositive for antineuronal antibodies and had elevated blood mercury as well. Another three autistic children (7.5%) had elevated blood mercury levels and negative results for antineuronal antibodies. Two other patients (5%) were seropositive for antineuronal antibodies and had normal levels of blood mercury. The remaining 10 patients (25%) were seronegative for antineuronal antibodies and had also normal levels of blood mercury.



**Figure 2.** Comparison between patients who were and were not seropositive for antineuronal antibodies in blood mercury levels. The boxes enclose the interquartile ranges (IQR) which are between the 25<sup>th</sup> and 75<sup>th</sup> percentiles. The horizontal line inside the box represents the median and the whiskers represent the non outlier or extreme maximum and minimum values. The closed small squares represent the extreme values (more than 3 IQR).

**Family history of autoimmune diseases in autistic and healthy children and its relation to seropositivity of antineuronal antibodies and blood mercury levels:**

Twenty out of the studied 40 autistic children (50%) had one or more, first or second-degree relative with an autoimmune disease [Rheumatoid arthritis (RA) in 12 patients, insulin-dependent diabetes mellitus (IDDM) in 3 patients, systemic lupus erythematosus (SLE) in 3 patients, autoimmune thyroiditis in one patient and rheumatic fever in one patient], 6 had mild to moderate autism and 14 had severe disease). Five out of the 20 autistic children with positive family history of autoimmune disease had a mother with

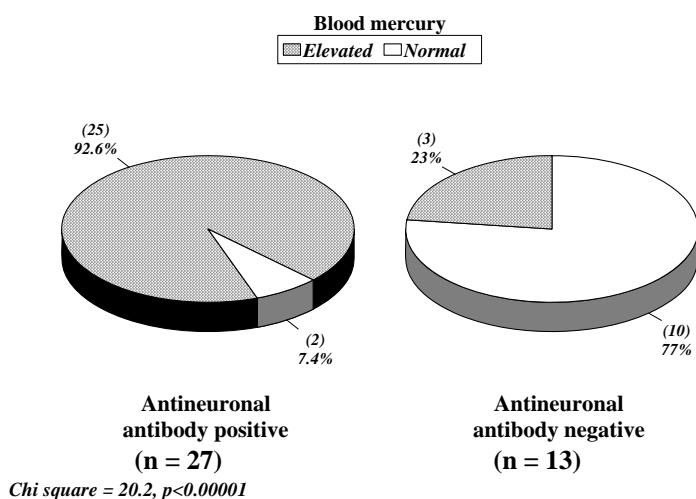
an autoimmune disease (2 had RA, one had SLE, one had IDDM and one had autoimmune thyroiditis). On the other hand, positive family history of autoimmune diseases was found in 4 only out of the studied 40 healthy children (10%) (RA in 2 children, SLE in one child and IDDM in one child). Thus, the frequency of autoimmune diseases among families of autistic children was significantly higher than that of normal children ( $X^2 = 16, p < 0.0001$ ).

Autistic children with positive family history of autoimmune diseases had significantly higher percent seropositivity of antineuronal antibodies and blood mercury levels than those without such history (table 2).

**Important clinical findings in autistic children (not included in CARS) and their relation to antineuronal antibody seropositivity and blood mercury levels:**

Thirteen out of the studied 40 autistic children (32.5%) had autistic regression, 20 (50%) had MR (IQ below 70)<sup>20</sup>, 11 (27.5%) had behavioral abnormalities (self injury and/or aggression), 10 (25%) had disturbed sleep and 8 (20%) were macrocephalic (i.e., their skull circumference was more than 3SD above the normal values for age and sex)<sup>22</sup>.

Patients with autistic regression, subnormal intellectual function and behavioral abnormalities had significantly higher percent seropositivity of antineuronal antibodies and blood mercury levels than autistic patients without such manifestations. Although patients who had disturbed sleep and macrocephaly had higher percent seropositivity of antineuronal antibodies and blood mercury levels than those without such clinical findings, yet these differences did not reach statistical significance.



**Figure 3.** Association between seropositivity of antineuronal antibodies and elevated blood mercury in autistic children.

**Table 2.** Seropositivity of antineuronal antibodies and blood mercury levels in relation to family history of autoimmunity and important clinical findings (not included in the CARS) in autistic children.

	Antineuronal antibodies			Blood mercury (ug/dl)		
	Seropositive (n =27)	Seronegative (n =13)	X <sup>2</sup> (p)	Mean±SD	Median(IQR)	Z (p)
Family history of autoimmunity						
Percent (n=20)	19 (95%)	1 (5%)	13.8	30.25±11.5	31 (17.25)	4.95
Absent (n=20)	8 (40%)	12 (60%)	(<0.0001)**	9.4±6.1	5 (12.5)	(<0.0001)**
Autistic regression						
Present (n=13)	12 (92.3%)	1 (7.7%)	5.4	28±13.6	23(21.5)	2.8
Absent (n=27)	15 (55.6%)	12 (44.4%)	(<0.05)*	15.9±12.5	16 (18)	(<0.01)**
Mental retardation						
Present (n=20)	18 (90%)	2 (10%)	9.2	27.85±13.3	29 (19.25)	3.8
Absent (n=20)	9 (45%)	11 (55%)	(<0.01)**	11.8±9.2	6.5 (13)	(<0.0001)**
Behavioral abnormalities						
Present (n=11)	10 (90.9%)	1 (9.1%)	3.8	27.9±14.3	23 (22)	2.4
Absent (n=29)	17 (58.6%)	12 (41.4%)	(<0.05)*	16.76±12.7	18 (20.5)	(<0.05)*
Sleep disturbance						
Present (n=10)	8 (80%)	2 (20%)	0.95	24.4±15.7	22.5 (28.75)	1.3
Absent (n=30)	19 (63.3%)	11 (36.7%)	(>0.05)	18.3±13.2	18 (24)	(>0.05)
Macrocephaly						
Present (n=8)	7 (87.5%)	1 (12.5%)	1.8	25.9±13.6	23 (21.5)	1.5
Absent (n=32)	20 (62.5%)	12 (37.5%)	(>0.05)	15.9±12.5	16 (18)	(>0.05)

p > 0.05: non significant, p < 0.05\*: significant, p < 0.01, 0.0001\*\*: highly significant

**EEG abnormalities in autistic children and their relation to seropositivity of antineuronal antibodies and blood mercury levels:**

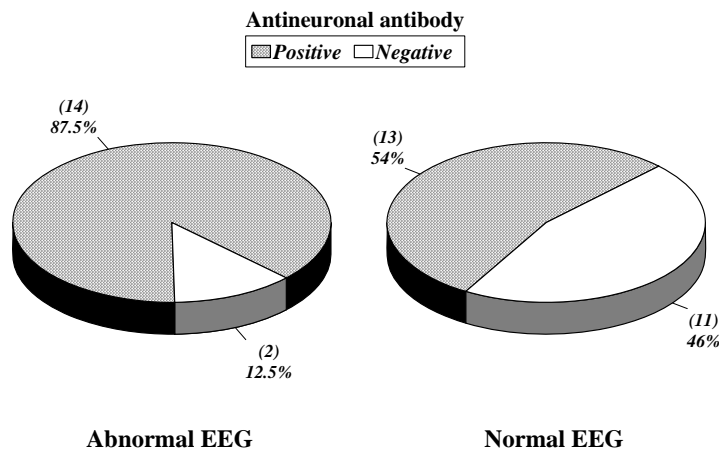
Sixteen out of the studied 40 autistic children (40%) had subclinical EEG abnormalities (focal abnormalities in 10 patients and diffuse abnormalities in the other 6 patients), 4 had mild to moderate and 12 had severe autism.

Autistic children with abnormal EEG had significantly higher percent seropositivity of antineuronal antibodies than patients with normal EEG (Figure 4). Similarly, the former group had significantly higher blood mercury levels [mean±SD = 27.5±14.7, median (IQR) = 23 (21.5)

µg/dL] than the latter group [mean±SD = 14.7±11, median (IQR) = 12.5 (13.75) µg/dL]. (Figure 5).

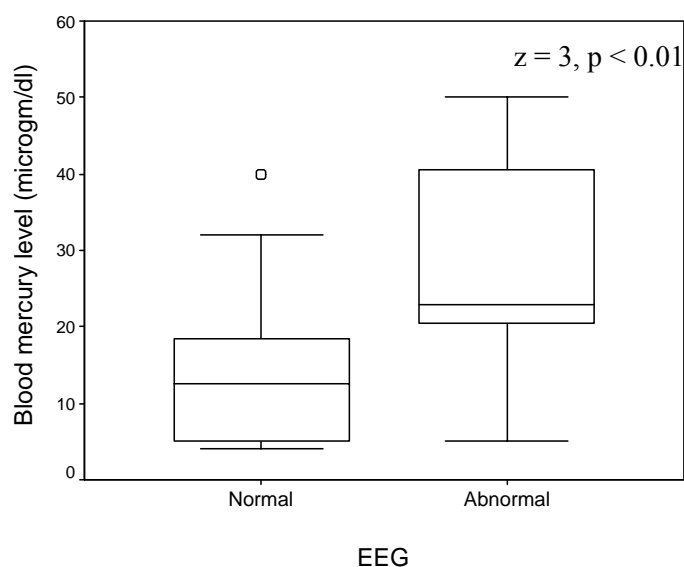
Although female autistic patients had higher percent seropositivity (8/9: 88.9%) of antineuronal antibodies than male autistic children (19/31: 61.3%), yet this difference did not reach statistical significance (p>0.05). Also, male and female autistic children had comparable blood mercury levels (p>0.05).

Blood mercury levels did not correlate significantly with the age of autistic children (p>0.05). In addition, none of autistic children had either rheumatic complains or clinical neurological findings.



**Figure 4.** Comparison between autistic patients with and without EEG abnormalities in seropositivity of antineuronal antibodies.

Chi square = 4.86, p<0.05



**Figure 5.** Comparison between autistic patients with and without EEG abnormalities in blood mercury levels. The small open squares represent the outlier values (between 1.5 and 3 IQR).

## DISCUSSION

Allergic autoimmune reaction to CNS, evidenced by the presence of brain autoantibodies, after exposure to mercury may play a causal role in some autistic children<sup>11</sup>. In our series, autistic children had significantly higher percent seropositivity of antineuronal antibodies (67.5%) than healthy controls (5%). To our knowledge, this is the first study to measure serum antineuronal antibodies in autistic children. Some patients with NPSLE and pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection (Tourette syndrome and OCD) were seropositive for antineuronal antibodies<sup>16,17</sup>. These antibodies bind to the surface membranes of neurons causing direct cytotoxic neuronal injury. The major autoantigens of antineuronal antibodies are unidentified<sup>16</sup>.

Searching for a mechanism underlying autoimmunity to brain in autism, blood mercury levels were assessed. Our results revealed significant elevation of blood Hg levels in autistic children compared to healthy controls. This study is one of the few to investigate the relationship between autism and mercury. In a recent study, elevated urinary coproporphyrin excretion, as an indicator of Hg toxicity, was reported in 83% of the studied autistic children<sup>23</sup>. Other investigators reported significant increase of the in-hair concentration levels of Hg in autistic patients compared to healthy children<sup>24</sup>. In another study, although blood mercury levels of autistic patients were higher than healthy children, the difference did not reach statistical significance<sup>5</sup>. Additional research should be conducted to evaluate the potential role for Hg exposure in autism.

The main reason behind the elevated blood mercury in autistic children is metallothionin (MT) dysfunction resulting from genetic polymorphism<sup>4</sup>. MT is a family of proteins bind to toxic chemicals allowing the body to eliminate them<sup>25</sup>. Autistic children can not adequately up-regulate MT biosynthesis following mercury exposure. Cultured lymphocytes from these patients challenged with thimerosal can not respond with an impressive up-regulation of MT<sup>26</sup>. In vitro, Hg and thimerosal levels found several days after vaccination inhibit methionin synthetase (MS) by 50%. Normal function of MS is crucial in biochemical steps necessary for brain development, attention and production of glutathione which is an important antioxidative agent. This potential deficit of Hg detoxification capacity coupled with the observed increase in autism in the last decade which parallels cumulative Hg exposure, has led to the suggestion that autism may be, in part, caused by environmental Hg exposure<sup>4</sup>. Some have proclaimed that chelation therapy for suspected Hg poisoning cures autistic children with high Hg levels. Hence, a well designed control study would be necessary to delineate the issue and alleviate the worrying of parents of autistic children<sup>5</sup>.

In the present work, there was a significant positive association between seropositivity of antineuronal antibodies and elevated blood Hg as 92.6% of the patients who were seropositive for antineuronal antibodies had also elevated blood Hg. In addition, 77% of the patients who were seronegative for antineuronal antibodies had normal blood Hg levels as well. This finding together with the significant elevation of blood Hg levels in

antineuronal antibodies seropositive compared to seronegative autistic children may point out to the possible role of Hg in induction of the production of antineuronal antibodies. Previous investigators reported that Hg and infectious agents are the two main environmental triggers for autoimmunity in autism<sup>10</sup>.

The following chain of events may lead to the production of brain autoantibodies (e.g., antineuronal antibodies) secondary to exposure to environmental triggers for autoimmunity (e.g., Hg) which may result in the development of autism; first; pre existing autoreactive T cells are generated by molecular mimicry as a result of contact with Hg, dietary proteins, and microbial antigens, with sequence homologies with autoantigens. Second; toxic chemicals, such as heavy metals (Hg) and viral antigens may increase adhesion molecules on brain endothelial cells. Third; pre existing autoreactive T cells may transmigrate across the blood brain barrier (BBB) and induce activation of local antigen presenting cells, such as microglia and astrocytes. Lastly; production of cytokine by T helper-1 autoreactive cells and the antigen presenting cells may result in oligodendrocyte damage and demyelination. As a result of this sequence of events neuronal antigens are released from neurofilaments and enter the circulation, resulting in immune reactions, such as the formation of plasma cells with the capacity of producing IgG, IgM, and IgA antibodies against neuron specific antigens. These antibodies may cross the BBB and combine with brain tissue antigens forming immune complexes, thus further damaging the neurological tissue<sup>27</sup>. Besides induction of autoimmunity, Hg could induce brain damage by other mechanisms which include depletion of antioxidants<sup>28</sup>, damage of mitochondria with subsequent robbing the cells of energy<sup>29</sup> and disruption of important neurotransmitters (such as serotonin, acetylcholine, glutamate and dopamine). All the previous abnormalities have been found in autism<sup>29,30</sup>.

In our series, children with severe autism had significantly higher percent seropositivity of antineuronal antibodies and blood Hg levels than those with a disease of mild to moderate severity. This finding together with the presence of a significant positive association between elevated blood mercury and seropositivity of antineuronal antibodies may point out to the presence of causal relationship between autistic severity and both antineuronal antibodies and blood Hg. This means that the higher was the blood mercury level, the more was the production of antineuronal antibodies

resulting in a more brain damage with a subsequent more severe disease.

To further understand if autoimmunity could play a role in autism, we studied the frequency of autoimmune diseases in families of autistic patients in comparison to healthy children. The frequency of autoimmune disease among families of the former group (50%) was significantly higher than that of the latter group (10%). Previous research has also found an increased frequency of autoimmunity in families of autistic children compared with those of healthy and autoimmune control subjects<sup>13</sup>. Thus, this may be an outstanding feature among autistic patients that points to their autoimmune background; the target in this case being the developing brain. We found a more significant increase of percent seropositivity of antineuronal antibodies and blood Hg levels in autistic patients with family history of autoimmunity than those without such history. This implies that in some families immune dysfunction, perhaps induced by certain environmental triggers as mercury, could express itself in the form of autism in one of its offspring.

Interestingly, autistic children with mental retardation and those with abnormal EEG had significantly higher percent seropositivity of antineuronal antibodies and blood Hg levels than patients without such abnormalities. These results denoted that antineuronal antibodies and blood Hg could induce brain damage in autistic children with subsequent decrease of mental power and increase in the EEG abnormalities. In addition, our study revealed a more significant increase of percent seropositivity of antineuronal antibodies and blood Hg levels in patients with regressive than those with non-regressive autism. This finding also supports the possible role of Hg as an environmental trigger of autoimmunity to brain in a normally developing child, whether genetically predisposed or not. The result is an immune mediated inflammatory reaction that culminates in the autistic phenotype.

In conclusion, Autism may be, in part, one of pediatric autoimmune neuropsychiatric disorders. This autoimmunity may be triggered by environmental Hg exposure. Further studies are warranted to shed light on the etiopathogenic role of antineuronal antibodies and mercury in autism. If this could be proved, routine assessment of serum antineuronal antibodies and blood mercury in autistic children will be mandatory. Furthermore, studies assessing the role of immunotherapy and Hg chelators as new therapeutic implications for autism are recommended.



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