

The Possible Association between Elevated Levels of Blood Mercury and the Increased Frequency of Serum Anti-myelin Basic Protein Auto-antibodies in Autistic Children

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Abstract:

Background: Autism can occur as a result of a complex interaction between environmental factors and genetic predisposition. Mercury is a neurotoxicant and it is one of the main environmental triggers for autoimmunity. The underlying pathogenic mechanism in autoimmune disorders is the formation of auto-antibodies. Brain specific auto-antibodies are elevated in a subgroup of autistic children. We are the first to study the relation between blood mercury levels and the seropositivity of anti-myelin basic protein (anti-MBP) autoantibodies in autistic children.

Methods: Blood mercury levels were measured, by atomic absorption spectrometry, and serum levels of anti-MBP auto-antibodies were measured, by ELISA, in 100 children with autism aged between 5-12 years and 100 healthy-matched control children.

Results: Serum levels of blood mercury were significantly higher in autistic children than healthy controls ($P<0.001$). Increased levels of blood mercury were found in 48% of autistic patients. In addition, 72% of autistic children had positive results of serum anti-MBP auto-antibodies. There was a significant positive association between the elevated levels of blood mercury and the positivity of serum anti-MBP auto-antibodies in autistic children ($P<0.001$).

Conclusions: Blood mercury levels were elevated in some autistic children and they were significantly associated with the production of serum anti-MBP auto-antibodies in a group of autistic children. Further research is warranted to determine if the production of brain auto-antibodies is triggered by environmental mercury exposure in autistic children. The possible therapeutic role of mercury chelators in autistic children should be also studied.

Keywords: Anti-myelin basic protein (anti-MBP) autoantibodies; Autism; Autoimmunity; Mercury

Introduction

In view of the possible multifactorial cause, autism can occur as a result of environment neurotoxicant heavy metals exposure such as mercury and lead in presence of genetic predisposition in some autistic children [1-5]. Early mercury exposure may result in neurological injury that may lead to developmental defects, including autism [6]. Several sources of toxic mercury exposure in children have been reported in literature. Mercurials may be found in various drugs, bleaching creams, antiseptics, disinfectants, as preservatives in cosmetics, tooth pastes, lens solutions, vaccines, contraceptives and immunotherapy solutions, fungicides, herbicides and in dental fillings, as well as in fish such as tuna due to water pollution. Mercury can cause immune, sensory, neurological, motor, and behavioral dysfunction similar to those associated with autism [7]. With increases in the environmental mercury exposure, there was a significant increase in the rates of autism and special education students. On average, for each 1,000 lb increase of the environmental mercury exposure, there was 61% increase in the rate of autism and 43%

increase in the rate of special education students. Thus, a logic step is to identify the source of mercury exposure in the child population and consider prevention and control of environmental pollution [8].

The presence of auto-antibodies to neural tissues/antigens in autism [1,9-15] and the increase in the frequency of autoimmune disorders among autistic families [16-20] suggest that autoimmunity may play an important role in the pathogenesis of autism in a subgroup of autistic children [1].

Mercury has been shown to induce proliferation and cytokine production from T lymphocytes [21]. Mercuric chloride in non-toxic doses induces the release of histamine and cytokines, such as IL-4 and tumor necrosis factor-alpha, from a murine mast cell line and from mouse bone marrow-derived cultured mast cells [22]. Mercury may be one of the main candidate environmental triggers for autoimmunity in autism, as it binds to lymphocyte receptors and/or tissue enzymes resulting in autoimmune reaction [23,24].

This study was the first to study the relation between whole blood mercury levels and seropositivity of anti-myelin basic protein (anti-MBP) autoantibodies in autistic children.

Materials and Methods

Study population

This cross-sectional study was conducted on 100 autistic children. They were recruited from the Pediatric Neuropsychiatric Clinic, Faculty of Medicine of Ain Shams University, Cairo, Egypt, during their follow up visits. Patients were fulfilling the criteria of the diagnosis of autism according to the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders [25]. The autistic group comprised 78 males and 22 females. Their ages ranged between 5 and 12 years (mean \pm SD=8.1 \pm 1.7 years). Patients who had associated neurological diseases (such as cerebral palsy and tuberous sclerosis), metabolic disorders (eg. Phenylketonuria), allergic manifestations or concomitant infection were excluded from the study.

The control group comprised 100 age- and sex- matched apparently healthy children. They included 77 males and 23 females. They were recruited from the Outpatients Clinic, Children's Hospital, Faculty of Medicine, Ain Shams University. They were the sibs of the children attending this clinic because of a minor illness (e.g common cold, tonsillitis and acute bronchitis). The control children were not related to the children with autism, and demonstrated no clinical findings suggestive of infections, allergic manifestations and immunological or neuropsychiatric disorders. Their ages ranged between 5 and 12 years (mean \pm SD=8.3 \pm 1.6 years).

The local Ethical Committee of the Faculty of Medicine, Ain Shams University, Cairo, Egypt approved this study. In addition, an informed written consent of participation in the study and its publication was signed by the parents or the legal guardians of the studied subjects.

Study measurements

Clinical evaluation of autistic patients: This was based on clinical history taking from caregivers, clinical examination and neuropsychiatric assessment. In addition, the degree of the disease severity was assessed by using the Childhood Autism Rating Scale [26].

Blood sampling

Two mililiters of venous blood were collected and transferred into a dry dean tube and left to dot 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 anti-MBP antibodies. Another one ml of blood was collected in a heparinized syringe for immediate assay of blood mercury.

Assessment of blood mercury levels

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 95th percentile of the control values which was 6 μ g/dl). To increase accuracy, all samples were analyzed twice in two independent experiments to assess inter-assay variations and to ensure reproducibility of the observed results (P>0.05).

Assessment of serum anti-myelin basic protein (anti-MBP) auto-antibodies: This was done by using ELISA kit that allows for the specific measurement of human anti-MBP (Diagnostic Systems, Texas, USA). This assay recognizes recombinant and natural human MBP antibodies. Principally, the microtiter plate provided in this kit has been precoated with MBP protein. Samples are added to the appropriate microtiter plate wells and incubated. Horseradish Peroxidase (HRP) conjugated to anti-antibody is added to each microplate well and incubated. Finally TMB (3,3',5,5'-tetramethylbenzidine) substrate solution is added to each well. Only those wells that contain anti-MBP and enzyme-conjugated anti-antibody exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wave length of 450 nm \pm 2 nm. To increase accuracy, all samples were analyzed twice in two independent experiments to assess inter-assay variations and to ensure reproducibility of the observed results (P>0.05). No significant cross-reactivity or interference was observed.

Statistical analysis

The results were analyzed by using the commercially available software package (Statview, Abacus concepts, inc., Berkley, CA, USA). The data were parametric, thus they were presented as mean \pm SD. Student's t-test was used for comparison between these data. Chi-square test was used for comparison between qualitative variables of the studied groups. Spearman's rho correlation coefficient "r" was used to determine the relationship between different variables. For all tests, a probability (P) of less than 0.05 was considered significant. Autistic patients were considered to have elevated blood mercury if their levels were above the chosen highest cut-off value which was 6.2 μ g/dl (mean \pm 2SD of the control values).

Results

Blood levels of mercury in autistic children and healthy control children

Blood levels of mercury were significantly higher in autistic children than healthy control children, P<0.001 (Table 1). Forty eight autistic children (48%) had increased blood mercury levels.

	Autistic patients (n=100)	Healthy controls (n=100)	P-value
	Mean \pm SD Range	Mean \pm SD Range	
Blood Mercury Levels (μg/dl)	18.7 \pm 6.2 3.2-32.4	4.3 \pm 1.4 1.2-6.5	0.001

Table 1: Levels of blood mercury in autistic patients and healthy children

There were no significant correlations between serum levels of blood mercury levels and the age of autistic children, $P>0.05$.

Positive results of serum anti-MBP auto-antibodies in autistic children and healthy control children.

Seventy two autistic children (72%) had positive results of serum anti-MBP auto-antibodies. Autistic children had significantly higher percent positivity of serum anti-MBP antibodies than healthy controls (5%), $P<0.001$.

The association between increased serum levels of blood mercury levels and the positive results of serum anti-MBP auto-antibodies

Autistic patients with increased blood mercury levels had significantly higher frequency of positive results of serum anti-MBP auto-antibodies (91.6%) than patients with normal blood mercury levels (53.8%), $P<0.001$ (Table 2).

	Autistic children with increased blood mercury levels (n=48)	Autistic children with normal blood mercury levels (n=52)	X2 (p)
Positive anti-MBP antibodies (n=72)	44 (91.6%)	28 (53.8%)	15.88
Negative anti-MBP antibodies (n=28)	4 (8.4%)	24 (46.2%)	(<0.001)

Table 2: The frequency of autistic patients with increased blood mercury levels in relation to the positivity of serum anti-MBP auto-antibodies.

Thus, there was a significant positive association between the elevated levels of blood mercury and the positivity of serum anti-MBP auto-antibodies in autistic children.

The results of blood mercury levels were quantitative. In contrast, the results of serum anti-MBP antibodies were qualitative (i.e either positive or negative) in autistic patients and healthy children. So, we couldn't calculate the correlation between the levels of blood mercury and serum anti-MBP antibodies.

Discussion

Because of the observed increase in the incidence of autism in the last decades, which parallels cumulative mercury exposure, it was proposed that autism may be caused by environmental mercury exposure in some children. It was hypothesized that children with autism have a decreased detoxification capacity due to genetic polymorphism [27]

In this work, blood levels of mercury were significantly higher in autistic children than healthy control children, $P<0.001$. In addition, 48% of autistic children had increased blood mercury levels. Previous studies also reported elevated mercury levels in the blood [3-5,7,8] and hair of some autistic children [2,28]. In addition, elevated urinary coproporphin excretion, which is an indicator of mercury toxicity, was reported in 83% of autistic children [29].

The main reason behind the elevated blood mercury levels in some autistic children may be metallothionin dysfunction resulting from genetic polymorphism. Mt is a family of proteins bind to toxic

chemicals allowing the body to eliminate them [30]. Autistic children cannot adequately up-regulate metallothionin biosynthesis following mercury exposure. Repetitive doses of thimerosal leads to neurobehavioral deteriorations in autoimmune susceptible mice, increased oxidative stress and decreased intracellular levels of glutathione in vitro. Subsequently, autistic children have significantly decreased level of reduced glutathione [27]. In addition, autistic patients were described as poor detoxifiers with remarkably less active glutathione-transferase which is important for the detoxification of mercury [4].

Promising treatments of autism may involve detoxification of mercury. Some have proclaimed that chelation therapy for suspected mercury poisoning may have a role in the treatment of autistic children with high mercury levels [27]. A significant decline in the blood levels of mercury and lead with the use of DMSA, as a chelating agent, has been reported. In addition, there was decline in the autistic symptoms with the decrease in the mercury and lead levels in blood after the use of DMSA [3].

The possible role of mercury used as preservative in vaccines [7] has been debated extensively, but most epidemiological studies do not support a causal association between vaccines and autism [31]. However, 87% of children included in the US Vaccine Adverse Event Reporting System had autism [32]. Moreover, a paper, based on computerized medical records in the Vaccine Safety Data-link, reported significantly increased rates of autism with mercury exposure from thimerosal-containing vaccines [33]. Although a measurable number of epidemiological studies have been conducted to clarify the associations between mercury exposure during embryonic life or early infancy and later incidences of autism or attention-deficit hyperactivity disorder, the conclusion still remains unclear [34].

Autoimmunity to CNS may have a pathogenic role in autism [1]. This may be indicated by the presence of brain-specific auto-antibodies in some autistic children [9-15]. In the current study, 72% of autistic children had positive results of serum anti-MBP auto-antibodies. Autistic children had significantly higher percent positivity of serum anti-MBP antibodies than the healthy controls (5%), $P<0.001$. Previous studies reported seropositivity of anti-MBP auto-antibodies in a subgroup of autistic children [35-37]. In one study, the seropositivity of anti-MBP protein auto-antibodies was reported in 58% of autistic children which was significantly higher than the control children (9%) [35]. A more recent study reported positivity of serum anti-MBP auto-antibodies in 80% of a group of 50 Saudian children with autism [12]. MBP is a protein believed to be important in the process of myelination of nerves in the central nervous system. Interest in MBP has centered on its role in demyelinating diseases, particularly multiple sclerosis. Several studies have shown a role for antibodies against MBP in the pathogenesis of multiple sclerosis [38,39].

The current study revealed that autistic patients with increased blood mercury levels had significantly higher frequency of positive results of serum anti-MBP auto-antibodies (91.6%) than patients with normal blood mercury levels (53.8%), $P<0.001$. Thus, there was a significant positive association between the elevated levels of blood mercury and the positivity of serum anti-MBP auto-antibodies in autistic children. This is the first study that investigated the relationship between increased blood mercury levels and serum levels of anti-MBP auto-antibodies in autism.

Allergic autoimmune reaction after exposure to heavy metals such as mercury may play a causal role in autism [23]. Mercury and infectious agents may be the two main environmental triggers for autoimmunity in autism [24]. Mercury has been shown to induce proliferation and cytokine production from T lymphocytes [21]. Mercury may be one of the main candidate environmental triggers for autoimmunity in autism, as it binds to lymphocyte receptors and/or tissue enzymes resulting in autoimmune reaction [23,24].

The following chain of events may lead to the production of brain autoantibodies secondary to exposure to environmental triggers for autoimmunity such as mercury exposure in autistic children. First; pre existing autoreactive T cells are generated by molecular mimicry as a result of contact with mercury, dietary proteins, and microbial antigens, with sequence homologies with autoantigens. Second; toxic chemicals, such as heavy metals 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 neurofilament 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, that further damage the neurological tissue [37].

Mercury is known to be neurotoxic, but its effects on the immune system are less well known. Mercury stimulates vascular endothelial growth factor and IL-6 release from human mast cells. This phenomenon could disrupt the blood-brain-barrier and permit brain inflammation. As a result, low levels of mercury may contribute to autism pathogenesis. Mast cells are involved in allergic reactions, but also in innate and acquired immunity, as well as in inflammation. Many patients with autism have allergic symptoms. Moreover, the prevalence of autism in patients with mastocytosis, characterized by numerous hyperactive mast cells in most tissues, is 10-fold higher than the general population suggesting mast cell involvement [40]. Besides its possible role in the induction of autoimmunity to CNS, mercury could induce brain damage by other mechanisms which include depletion of glutathione and other antioxidants, damage of mitochondria with subsequent depletion of the cells of energy and disruption of important neurotransmitters such as serotonin, acetylcholine, glutamate and dopamine. All the previous abnormalities have been found in autism [41-43].

This study revealed that the increase of blood mercury levels may promote the induction of autoimmunity through stimulation of the production of brain auto-antibodies. As this study is the first that investigated the relationship between blood mercury levels and serum anti-MBP auto-antibodies in autistic, we recommend future studies that relate the elevated levels of blood mercury and other heavy metals and the production of brain specific auto-antibodies in autistic children.

Conclusions

Blood mercury levels were elevated in some autistic children and they were significantly associated with the production of serum anti-MBP auto-antibodies in a group of autistic children. Further research

is warranted to determine if the production of brain auto-antibodies is triggered by environmental mercury exposure in autistic children. The possible therapeutic role of mercury chelators in autistic children should be also studied.

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