Metab Brain Dis
DOI 10.1007/s11011-016-9836-8

ORIGINAL ARTICLE

The positive association between elevated blood lead levels and brain-specific autoantibodies in autistic children from low lead-polluted areas

Gehan Ahmed Mostafa[1,2] · **Geir Bjørklund**[3] · **Mauricio A. Urbina**[4] · **Laila Yousef Al-Ayadhi**[2]

Received: 19 March 2016 / Accepted: 16 May 2016

Abstract The underlying pathogenic mechanism in autoimmune disorders is the formation of autoantibodies. In children with autism spectrum disorder (ASD), it has been documented increased levels of brain-specific autoantibodies. Furthermore, lead (Pb) has been identified as one of the main neurotoxicants acting as environmental triggers for ASD as it induces neuroinflammation and autoimmunity. The present study is the first to explore a potential relationship between the levels of blood lead (BPb) and seropositivity of anti-ribosomal P protein antibodies in ASD children. Levels of BPb and serum anti-ribosomal P protein antibodies were measured in 60 children with ASD and 60 healthy control matched children, aged between 5 and 12 years, recruited from low Pb-polluted areas. The levels of BPb were significantly higher in ASD children than in healthy control children ($P < 0.001$). Patients with ASD had significantly higher frequency of increased BPb levels ≥10 μg/dL (43.3 %) than healthy control children (13.3 %; $P < 0.001$). There were significant and positive correlations between the levels of BPb, and the values of Childhood Autism Rating Scale (CARS) ($P < 0.01$) and IQ in children with ASD ($P < 0.001$). Patients with ASD showing increased levels of BPb had significantly higher frequency of seropositivity of anti-ribosomal P antibodies (92.3 %) than patients with normal BPb levels (32.3 %; $P < 0.001$). The findings of the present study suggest that increased levels of BPb in some children with ASD may trigger the production of serum anti-ribosomal P antibodies. Further research is warranted to determine if the production of brain autoantibodies is triggered by environmental Pb exposure in children with ASD. The possible therapeutic role of Pb chelators in ASD children should also be studied.

Keywords Anti-ribosomal P protein antibodies · Autism · Autoimmunity · Blood lead · Cognitive function

✉ Gehan Ahmed Mostafa
gehan.mostafa2000@yahoo.com

[1] Department of Pediatrics, Faculty of Medicine, Ain Shams University, Cairo, Egypt

[2] Department of Physiology, Autism Research and Treatment Center, AL-Amodi Autism Research Chair, Faculty of Medicine, King Saud University, Riyadh, Saudi Arabia

[3] Council for Nutritional and Environmental Medicine, Mo i Rana, Norway

[4] Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile

Introduction

The etiology of autism spectrum disorder (ASD) is likely multifactorial, with research showing that ASD is caused by the interplay of genes and environmental factors (Vojdani et al. 2003; Cohly and Panja 2005). Lead (Pb) pollution is a substantial problem in developing countries. Lead is an element with unknown physiological function in humans, but adversely affects a variety of fundamental biochemical processes (Matte 2003). The US Centers for Disease Control and Prevention (CDC) defines blood Pb (BPb) concentration as "elevated" or "level of concern" above 10 μg/dL (CDC 2007). This value has become crucial as policymakers and public health officials have acted to remove sources of Pb exposure only after the CDC and Prevention's level of concern had been exceeded. Despite this efforts, it has been shown that even BPb concentrations below 10 μg/dL are inversely associated with children's intelligence quotient (IQ) scores at three and five years of age (Canfield et al. 2003; AAP 2005). As a result of these findings, the CDC has

Published online: 01 June 2016

recommended decreasing the BPb threshold down to 5 μg/dL, but advised not to use the term "level of concern" for this new threshold (CDC 2012a; b). Regardless of the cutoff values, no level of BPb is considered safe, particularly due to its adverse effects on children's neurodevelopment (Ha et al. 2009).

Lead belongs to the most toxic and insidious group of sulfhydryl-reactive metals because they disrupt many biochemical and nutritional processes (Quig 1998). Worldwide, six categories of products account for most cases of Pb exposure which include gasoline additives, food-can soldering, Pb-based paints, ceramic glazes, drinking water pipe systems, and ethnic over-the-counter medications. Lead may enter the body by ingestion through the intestines, by inhalation through the lungs or by absorption through the skin. Children are more vulnerable to Pb exposure than adults because of the frequency of pica and hand-to-mouth activity. Also, children absorb 40–50 % of dietary Pb, whereas adults absorb only 5–10 % (Markowitz 2000).

Autoimmunity may play a role in a subgroup of ASD patients (Cohly and Panja 2005; Vojdani et al. 2003), as indicated by the presence of brain-specific autoantibodies in some ASD children (Al-Ayadhi and Mostafa 2012; Mostafa et al. 2008; Mostafa et al. 2010; Mostafa and Al-Ayadhi 2011; Singh and Rivas 2004; Singh et al. 1998; Vojdani et al. 2003). In immune-mediated neurological disorders, various antibodies against neuronal tissues have been discovered and its overexpression documented. In several cases, some of these antibodies have been found to be related with the pathological mechanism of the disease (Greenwood et al. 2002). Anti-ribosomal P protein antibodies are one group of potentially pathogenic auto-antibodies that has a specificity for the functional center of the ribosomal P proteins. These proteins are a family of highly conserved acidic phosphoproteins primarily located on the stalk of the large (60s) ribosomal subunit (Gerli and Caponi 2005). They bind three ribosomal proteins identified as P0, P1 and P2 (38, 19 and 17-kDa, respectively) by recognizing a certain epitope found in those three proteins. Some of the possible pathogenic mechanisms of these antibodies in some autoimmune diseases include their binding to epitopes on the cell membrane surface, intracellular penetration, inhibition of protein synthesis, production of pro-inflammatory cytokines and induction of cell apoptosis (Toubi and Shoenfeld 2007).

Allergic autoimmune reaction after exposure to heavy metals such as Pb and mercury (Hg) may play a causal role in ASD. Heavy metal exposure may be one of the main candidate environmental triggers for autoimmunity in ASD, as they bind to lymphocyte receptors and/or tissue enzymes resulting in an autoimmune reaction (Cohly and Panja 2005; Singh and Hanson 2006; Vojdani et al. 2003). The main aim of this study was to investigate a potential relationship between BPb levels and anti-ribosomal P protein antibodies in ASD children.

Methods

Study population

This cross-sectional study was conducted on 60 ASD children. They were recruited from the Pediatric Neuropsychiatric Clinic, Faculty of Medicine of Ain Shams University, Cairo, Egypt, during their follow-up visits. Patients met the criteria for the diagnosis of ASD according to the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders (APA 1994). The ASD group comprised 46 males and 14 females, with their ages ranging between 5 and 12 years old (mean ± SD = 9.7 ± 2.3 years). Patients who had associated neurological diseases (such as cerebral palsy and tuberous sclerosis), metabolic disorders (e.g., phenylketonuria), allergic or autoimmune manifestations or concomitant infection were excluded from the study.

The control group comprised 60 age- and sex (45 males, 15 females) - matched healthy children. Their ages also ranged between 5 and 12 years (mean ± SD = 9.6 ± 2.4 years). They were recruited from the Outpatients Clinic, Children's Hospital, Faculty of Medicine, Ain Shams University. They were the siblings 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 ASD, and demonstrated no clinical findings suggestive of infections, allergic manifestations, and immunological or neuropsychiatric disorders.

All studied children lived in areas with no obvious source of Pb pollution (Nasr City and Heliopolis). This study was approved by the local Ethical Committee of the Faculty of Medicine, Ain Shams University, Cairo, Egypt. Written consent for participation in the study and its publication was also obtained and signed by the parents or the legal guardians of the subjects.

Clinical evaluation

Clinical evaluation of patients with autism spectrum disorder was based on clinical history taken from caregivers, a clinical examination, and a neuropsychiatric assessment. In addition, the degree ASD severity was assessed by using the Childhood Autism Rating Scale (CARS) (Schopler et al. 1986) which rates the child on a scale from one to four in each of the 15 areas included (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 this scale, children who have scored 30–36 have mild to moderate ASD, while those scoring between 37 and 60 points have a severe degree of ASD.

Assessment of cognitive function

Assessment of cognitive function (memory, attention, language, concept formation, problem solving, executive and visuospatial functions) with age-appropriate, translated and validated psychometric instruments, were administered by well-trained psychologists using a set of Arabic norms (Meleka and Ismail 1999) for a translated Wechsler Intelligence Scale for Children, 3rd Edition (WISC-III) (Wechsler 1991). This scale is the most commonly used test to assess cognitive function in the children. Three global measures were examined in the present study, as follow. The verbal intelligence quotient (IQ), derived from different subtests including information, similarities, arithmetic, comprehension, vocabulary and digit span. The performance IQ derived from different subtests including picture completion, block design, picture arrangement, object assembly and digit symbol. And finally, the full-scale IQ, which is the sum of the verbal and performance IQ. The individual subtests may be particularly useful because each depends on a variety of capabilities, and dysfunction of any of these could result in a low score on one of the global measures. Cognitive dysfunction was diagnosed when the difference between the verbal and performance IQ was higher than 15, and/or when the result of one or more of the individual subtests was below seven, and/or the full-scale IQ was below 70.

Measurements of blood lead levels

Levels of BPb were measured by atomic absorption spectrophotometer (graphite-tube technique). Venous whole blood samples were diluted and analyzed on a PerkinElmer Elan DRC II inductively-coupled plasma mass spectrometer (PerkinElmer, Waltham, MA, USA). According to the Centers for Disease Control and Prevention (CDC 2007), children were categorized into those with BPb levels ≥10 μg/dL and those with levels <10 μg/dL.

Measurement of serum anti-ribosomal P protein antibodies

Serum total IgG and IgM anti-ribosomal P protein antibodies were measured by ELISA using ribosomal P peptide-bovine serum albumin conjugate as an antigen (Nunc Immuno-Module F8 MaxiSorp, Nalge Nunc International, Roskilde, Denmark). To increase accuracy, all samples were analyzed twice in two independent experiments to assess the inter-assay variation and repeatability 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 parametric data were presented as the mean and standard deviation (SD), while non-parametric data were presented as a median and interquartile range (IQR, between the 25th and 75th percentiles). Student's t-test was used for comparison of parametric data, while Mann-Whitney test was used for comparison between non-parametric 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.

Results

All studied ASD patients had classic-onset ASD. None of the ASD patients had regressive ASD, associated neurological diseases (such as cerebral palsy and tuberous sclerosis), metabolic disorders (e.g., phenylketonuria), allergic manifestations or concomitant infections. Based on CARS scoring, 34 patients had a mild to moderate ASD, while other 26 presented a severe degree of ASD. In addition, 31 ASD children had a subnormal intellectual function (intelligence quotient below 70), with 21 of them presenting mental retardation (intelligence quotient =50–69) and the remaining ten having moderate mental retardation (intelligence quotient =35–49). None of the healthy control children had a subnormal intellectual function (Table 1).

Blood levels of lead in ASD children and healthy control children

The levels of BPb ranged between 3 and 29 μg/dL in ASD children and between 2 and 12 μg/dL in healthy control children (Table 1). Blood levels of Pb were significantly higher in ASD children (median (IQR) = 9 (8) μg/dL) than in healthy control children (median (IQR) = 6 (3) μg/dL) ($P < 0.001$; Table 2).

Twenty-six (26) ASD children (43.3 %) had increased BPb levels ≥10 μg/dL, while only four healthy children (13.3 %) had increased BPb levels. ASD patients had significantly higher frequency of increased BPb levels than healthy control children ($P < 0.001$). Furthermore, patients with severe ASD had significantly higher BPb levels than patients with mild to moderate ASD ($P < 0.001$) (Table 2).

There was a significant and positive correlation between BPb levels and CARS scores in ASD children ($P < 0.001$; Fig. 1a). Also, BPb levels correlated significantly and inversely with IQ values in ASD children ($P < 0.001$; Fig. 1b). On the other hand, there were no significant correlations between BPb levels and the age of ASD children ($P > 0.05$).

Table 1 Demographic and laboratory data from ASD and healthy control children

	Children with ASD (n = 60)	Control group (n = 60)
Age (in years): Range	5–12	5–12
Mean ± SD	9.7 ± 2.3	9.6 ± 2.4
Sex: (Male/Female)	46/14	45/15
Intelligence quotient: Above 70	48.3 %	100 %
50–69	35 %	
35–49	16.7 %	
CARS scores: Mild to moderate (30–36)	56.7 %	
Severe (37–60)	43.3 %	
Blood lead (μg/dL): Range	3–29	2–12
Median (IQR)	9 (8)	6 (3)
Positivity of anti-ribosomal P antibodies	58.3 %	5 %

CARS, Childhood Autism Rating Scale; IQR, interquartile ranges

Serum anti-ribosomal P protein antibodies in ASD children and healthy control children

Children with ASD had significantly higher percent positivity of serum anti-ribosomal P antibodies than healthy controls ($P < 0.001$). Thirty-five (35) ASD children (58.3 %) had positive results of serum anti-ribosomal P antibodies, while only three healthy children showed positive results (5 %).

The association between increased serum levels of blood lead levels and the positive results of serum anti-ribosomal P antibodies

Levels of BPb ranged between 8 and 29 μg/dL in ASD with positive results of serum anti-ribosomal P antibodies, while BPb ranged only between 3 and 10 μg/dL in patients with negative results of these antibodies. Patients with ASD and positive results of serum anti-ribosomal P antibodies had significantly higher BPb levels (median (IQR) = 13 (8) μg/dL) than patients with negative results of these antibodies (median (IQR) = 6 (4) μg/dL) ($P < 0.001$; Fig. 2).

Table 2 Comparison of blood lead (BPb) levels in the studied children

Studied children	Blood lead levels (μg/dL) Median (IQR)	Z (P)
Children with ASD (n = 60)	9 (8)	4.9
Healthy children (n = 60)	6 (3)	(<0.001)
Mild to moderate ASD (n = 34)	7 (4)	5.9
Severe ASD (n = 26)	14 (8)	(<0.001)

IQR, interquartile ranges

In addition, ASD patients with increased BPb levels had significantly higher frequency of positive results of serum anti-ribosomal P antibodies (92.3 %) than patients with normal BPb levels (32.3 %) ($P < 0.001$; Table 3).

Discussion

Elevated BPb is recognized as an important factor associated with neurodevelopmental impairment, and physiological and behavioral disorders in children (Kordas et al. 2007). Particularly, Pb has been suggested as one of the main neurotoxicants considered as an environmental trigger for ASD (Vojdani et al. 2003; Cohly and Panja 2005). In this work, blood levels of Pb were significantly higher in ASD children than in healthy control children. Also, within the ASD patients, 43.3 % of them had increased BPb levels ≥10 μg/dL, while only four healthy children (13.3 %)

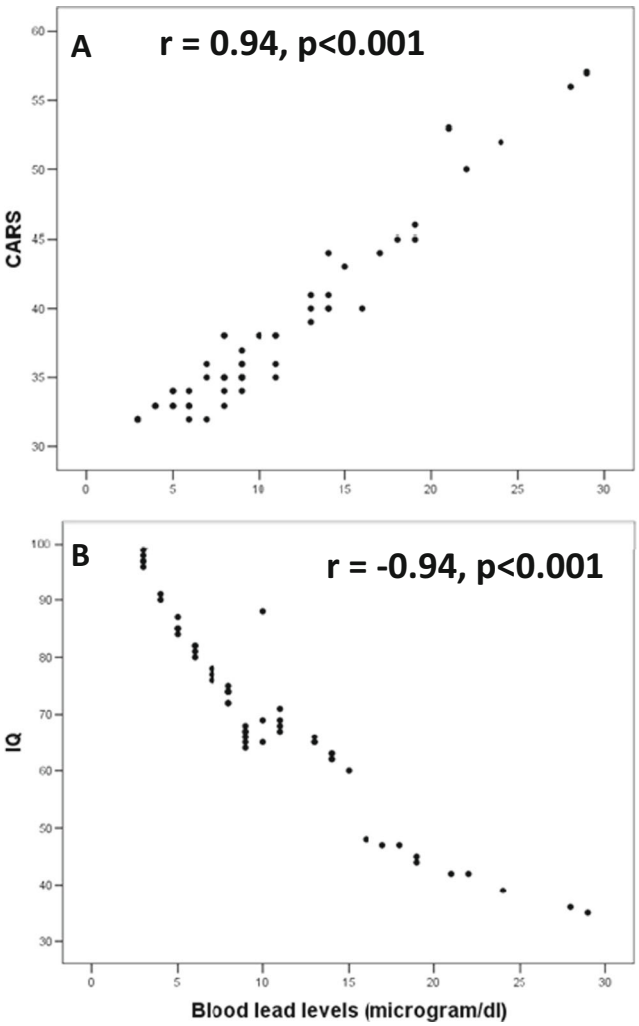

Fig. 1 Correlations between blood lead levels and Childhood Autism Rating Scale (CARS) and values of intelligence quotient (IQ) in ASD children

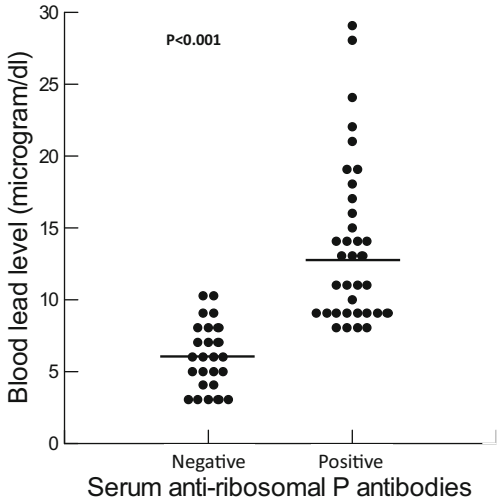

Fig. 2 Blood lead levels in relation to positivity of serum anti-ribosomal P antibodies in children with ASD. Horizontal bars represent the median values

presented elevated BPb levels. Patients with ASD had significantly higher frequency of increased BPb levels than healthy control children.

Several studies have investigated the possible association between exposure to Pb and ASD, but their findings are conflicting (Fido and Al-Saad 2005; Kern et al. 2007; Lakshmi Priya and Geetha 2010; Blaurock-Busch et al. 2011; Tian et al. 2011; Blaurock-Busch et al. 2012a; Adams et al. 2013; Alabdali et al. 2014). Higher levels of Pb in hair samples from ASD children compared to age- and sex-matched healthy controls have been reported (Lakshmi Priya and Geetha 2010). For example, a case-control study of 40 boys (4–8 years) with ASD and 40 non-affected age-matched typically developing boys from Kuwait, reported increased levels of Pb in the hair of children with ASD (6.75 μg/g) compared to controls (3.20 μg/g) (Fido and Al-Saad 2005). More recently, based on a case-control study in Saudi Arabia (25 children with ASD and 25 healthy children), Blaurock-Busch et al. (2011) reported similar results. On the other hand, another study from Saudi Arabia involving 52 children with ASD and 30 healthy controls between 3 and 12 years of age reported that ASD cases had a significantly higher mean red blood cell Pb concentration compared to healthy controls (6.79 μg/dL vs. 4.73 μg/dL, Alabdali et al. 2014). Similarly, Adams et al. (2013) reported that ASD cases had higher red blood cells Pb levels (mean = 19 μg/g in ASD cases vs. 13 μg/g in controls (Adams et al. 2013). In contrast, a study from Dallas (Texas) reported a significantly lower concentration of Pb in the hair of 45 children with ASD (ages 1–5 years) compared with 45 gender-, age- and race-matched controls (Kern et al. 2007). Furthermore, the Childhood Autism Risk from Genetics and Environment Study in California did not find any significant differences between mean BPb concentrations from 2 to 5-year-old children with ASD (n = 37) and healthy controls (n = 15) (Tian et al. 2011). This disparity in the results might be explained by differences in the severity of ASD in the subjects (see below).

The present study does not only revealed significantly higher BPb levels in ASD patients compared to healthy controls, but also found that patients with severe ASD had higher BPb levels than patients with mild to moderate ASD. Therefore, the present results do not only support the findings of previous studies suggesting that Pb levels are higher in ASD patients, but also might explain the disparity between studies. The present finding of that patients with severe ASD had higher BPb levels than patients with mild to moderate ASD suggest that studies comparing Pb levels between healthy children and mild to moderate ASD patients are less likely to find an effect compared to studies were patients with severe ASD were recruited. Also, there was a significant and positive correlation between BPb levels and the values of CARS in ASD children (P < 0.001). Similarly, Blaurock-Busch et al. (2012a) reported a significant association between Pb levels in hair and ASD severity scores in a study that involved 44 children with ASD from Saudi Arabia. This further indicates that the extent of the elevation of BPb levels is linked to the degree of ASD severity. However, it is not easy to determine whether the increase in BPb levels has a pathogenic role in the disease. The main reason for the elevated BPb and Hg levels in some ASD children may be metallothionein (MT) dysfunction resulting from genetic polymorphism. MT is a family of proteins that bind to toxic chemicals allowing the body to eliminate them (Lakshmi Priya and Geetha 2010). Children with ASD cannot adequately up-regulate MT biosynthesis following exposure to toxic chemicals. Also, ASD patients were described as poor detoxifiers with a remarkably lower activity of glutathione-transferase, crucial for the detoxification of toxic chemicals (Alabdali et al. 2014). One study suggested that children with ASD may have a reduced ability to detoxify heavy metals - and as a result, cannot excrete Pb from their bodies compared to healthy controls (mean Pb levels in urine =1.19 μg/g creatinine in ASD cases vs.

Table 3 The frequency of ASD patients with positive results of serum anti-ribosomal P antibodies in relation to BPb levels

	ASD children with elevated BPb (n = 26)	ASD children with normal BPb (n = 34)	X^2 (p)
Positive anti-ribosomal P antibodies (n = 35)	24 (92.3 %)	11 (32.3 %)	22.6
Negative anti-ribosomal P antibodies (n = 25)	2 (7.7 %)	23 (67.7 %)	(< 0.001)

4.63 μg/g creatinine in controls) (Yorbik et al. 2010). However, it is worth noting that none of these studies mentioned earlier adjusted their results to control for potential environmental exposures.

In the present study, BPb levels had a significant and inverse correlation with values of IQ in ASD children ($P < 0.001$), thus suggesting a significant association between BPb levels and cognitive function. One of the most deleterious outcomes of Pb exposure is a decline in cognitive functioning (memory, attention, language, concept formation, problem-solving, executive and visuospatial functions) which also has an impact on school performance (Kamel et al. 2003). Lead induce damage to the blood-brain barrier, causing a subsequent impairment of cognitive development (Zheng et al. 2003). Lead also inhibits important enzymes participating in the synthesis of heme proteins (Piomelli 2002). For example, anemia, leading to cerebral hypoxia has also been suggested to have a significant influence on cognition (Petranovic et al. 2008). Experimental studies have shown that Sulphur-containing antioxidants have beneficial effects against the detrimental oxidative stress induced by Pb (Caylak et al. 2008).

Autoimmunity to CNS may have a pathogenic role in ASD (Cohly and Panja 2005). In the current study, 58.3 % of ASD children had positive results of serum anti-ribosomal P antibodies, significantly higher than healthy controls (5 %). Seropositivity of anti-ribosomal P antibodies has been previously reported in 44.3 % and 40.6 % of 70 and 46 Saudi children with ASD, respectively (Mostafa and Al-Ayadhi 2011; Al-Ayadhi and Mostafa 2012).

Anti-ribosomal P protein antibodies are highly specific for systemic lupus erythematosus (SLE), in particular for the neuropsychiatric manifestations including psychosis, mood disorders, anxiety, cognitive dysfunction, and delirium. There are some studies in the literature relating anti-ribosomal P protein antibodies to the pathogenesis of organ damage in SLE. The main pathways described are cross-reaction with anti-dsDNA antibodies, a cytotoxic effect on mesangium cell proliferation, invasion into living cells and starting apoptosis, a defect in the synthesis of apolipoprotein B resulting in accumulation of lipids inside cells, and a downregulation of the total protein synthesis. P proteins are post-translationally modified (dephosphorylated) during apoptosis, and a dysregulation in the normal clearance of apoptotic cells leads to aberrant exposure of the immune system to modified nonself-antigens. This has been postulated as one of the potential triggers for the development of anti-P autoimmune response in some autoimmune diseases (Ben-Ami et al. 2010).

In the present study, ASD patients with positive results of serum anti-ribosomal P antibodies had significantly higher BPb levels than patients with negative results of these antibodies. Also, ASD patients with increased BPb levels had significantly higher frequency of positive results of serum anti-ribosomal P antibodies (92.3 %) than patients with normal BPb levels (32.3 %). Thus, there was a significant positive association between the elevated levels of BPb and the positivity of serum anti-ribosomal P antibodies in ASD children. This is the first study that investigated the relationship between increased BPb levels and serum levels of anti-ribosomal P antibodies in ASD patients.

Allergic autoimmune reaction after exposure to heavy metals such as Pb and Hg may play a causal role in ASD (Singh and Hanson 2006). Heavy metals exposure and infectious agents may be the two main environmental triggers for autoimmunity in ASD (Vojdani et al. 2003; Cohly and Panja 2005). The following chain of events may lead to the production of brain autoantibodies, secondary to exposure to environmental triggers for autoimmunity such as Hg exposure in ASD children. 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. Secondly, toxic chemicals, such as heavy metals and viral antigens may increase adhesion molecules in brain endothelial cells. Then, 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. Finally, production of cytokines by T helper-1 autoreactive cells and the antigen presenting cells may result in oligodendrocyte damage and demyelination. As a consequence of this the sequence of events, neuronal antigens are released from the neurofilament and enter the circulation, resulting in immune reactions, such as the formation of plasma cells with the capacity for 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, which further damage the neurological tissue (Vojdani et al. 2002; Vojdani et al. 2003).

Oral dimercaptosuccinic acid (DMSA) chelation increases the urinary output of toxic and neurotoxic metals. It acts by forming sulfhydryl linkages to divalent metal cations, forming a chelated metal complex which is excreted in the urine. Oral DMSA treatment is considered an efficient and safe chelating agent for use in children, and has been reported to have beneficial effects in ASD patients, resulting in significant improvements in verbal and nonverbal communication in these patients (Blaurock-Busch et al. 2012b). The possible therapeutic role of Pb chelators in ASD children should be further studied, as it seems to offers promising results.

The present study revealed that the increase in BPb levels may promote the induction of autoimmunity through stimulation of the production of brain auto-antibodies. As this is the first study that investigated the relationship between BPb levels and serum anti-ribosomal P antibodies in ASD, further attention should receive a potential relationship between elevated concentrations of BPb (and other heavy metals) and the production of brain-specific autoantibodies in ASD children.

Conclusions

Increased levels of BPb may trigger the production of serum anti-ribosomal P antibodies in some ASD children. Further research is needed to determine if the production of brain auto-antibodies is triggered by environmental Pb exposure in ASD children. The possible therapeutic role of Pb chelators in ASD children should receive further attention.

Acknowledgments This research project was supported by a grant from the Research Center of the Center for Female Scientific and Medical Colleges, Deanship of Scientific Research, King Saud University.

Compliance with ethical standards

Conflict of interest The authors declare no potential conflicts of interest with respect to the authorship, and/or publication of this article.

Ethical approval All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee, and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

AAP - American Academy of Pediatrics Committee on Environmental Health (2005) Lead exposure in children: prevention, detection, and management. Pediatrics 116:1036–1046

Adams JB, Audhya T, McDonough-Means S, Rubin RA, Quig D, Geis E, Gehn E, Loresto M, Mitchell J, Atwood S, Barnhouse S, Lee W (2013) Toxicological status of children with autism vs. neurotypical children and the association with autism severity. Biol Trace Elem Res 151:171–180

Alabdali A, Al-Ayadhi L, El-Ansary A (2014) A key role for an impaired detoxification mechanism in the etiology and severity of autism spectrum disorders. Behav Brain Funct 10:14. doi:10.1186/1744-9081-10-14

Al-Ayadhi LY, Mostafa GA (2012) A lack of association between elevated serum levels of S100B protein and autoimmunity in autistic children. J Neuroinflammation 9:54. doi:10.1186/1742-2094-9-54

APA - American Psychiatric Association (1994) Diagnostic and statistical manual of mental disorders, 4th edn. American Psychiatric Association, Washington

Ben-Ami SD, Blank M, Altman A (2010) The clinical importance of anti-ribosomal-P antibodies (in Hebrew). Harefuah 149(794–797):810

Blaurock-Busch E, Amin OR, Rabah T (2011) Heavy metals and trace elements in hair and urine of a sample of Arab children with autistic spectrum disorder. Maedica (Buchar) 6:247–257

Blaurock-Busch E, Amin OR, Dessoki HH, Rabah T (2012a) Toxic metals and essential elements in hair and severity of symptoms among children with autism. Maedica (Buchar) 7:38–48

Blaurock-Busch E, Amin OR, Dessoki HH, Rabah T (2012b) Efficacy of DMSA therapy in a sample of Arab children with autistic spectrum disorder. Maedica (Buchar) 7:214–221

Canfield RL, Henderson CR, Cory-Slechta DA, Cox C, Jusko TA, Lanphear BP (2003) Intellectual impairment in children with blood lead concentrations below 10 microg per deciliter. N Engl J Med 348:1517–1526

Caylak E, Aytekin M, Halifeoglu I (2008) Antioxidant effects of methionine, alpha-lipoic acid, N-acetylcysteine and homocysteine on lead-induced oxidative stress to erythrocytes in rats. Exp Toxicol Pathol 60:289–294

CDC - Centers for Disease Control and Prevention (2012a) CDC response to advisory committee on childhood lead poisoning prevention: recommendations in "low level lead exposure harms children: a renewed call of primary prevention". Centers for Disease Control and Prevention. http://www.cdc.gov/nceh/lead/acclpp/cdc_response_lead_exposure_recs.pdf. Accessed 7 March 2016

CDC - Centers for Disease Control and Prevention (2012b) Blood lead levels in children: What do parents need to know to protect their children? Centers for Disease Control and Prevention. http://www.cdc.gov/nceh/lead/acclpp/lead_levels_in_children_fact_sheet.pdf. Accessed 7 March 2016

CDC - Centers for Disease Control and Prevention Advisory Committee on Childhood Lead Poisoning Prevention (2007) Interpreting and managing blood lead levels <10 microg/dL in children and reducing childhood exposures to lead: recommendations of CDC's Advisory Committee on Childhood Lead Poisoning Prevention. MMWR Recomm Rep 56(RR-8):1–16

Cohly HH, Panja A (2005) Immunological findings in autism. Int Rev Neurobiol 71:317–341

Fido A, Al-Saad S (2005) Toxic trace elements in the hair of children with autism. Autism 9:290–298

Gerli R, Caponi L (2005) Anti-ribosomal P protein antibodies. Autoimmunity 38:85–92

Greenwood DL, Gitlits VM, Alderuccio F, Sentry JW, Toh BH (2002) Auto-antibodies in neuropsychiatric lupus. Autoimmunity 35:79–86

Ha M, Kwon HJ, Lim MH, Jee YK, Hong YC, Leem JH, Sakong J, Bae JM, Hong SJ, Roh YM, Jo SJ (2009) Low blood levels of lead and mercury and symptoms of attention deficit hyperactivity in children: a report of the children's health and environment research (CHEER). Neurotoxicology 30:31–36

Kamel NM, Ramadan AM, Kamel MI, Mostafa YA, Abo el-Naga RM, Ali AM (2003) Impact of lead exposure on health status and scholastic achievement of school pupils in Alexandria. J Egypt Public Health Assoc 78:1–28

Kern JK, Grannemann BD, Trivedi MH, Adams JB (2007) Sulfhydryl-reactive metals in autism. J Toxicol Environ Health A 70:715–721

Kordas K, Casavantes KM, Mendoza C, Lopez P, Ronquillo D, Rosado JL, Vargas GG, Stoltzfus RJ (2007) The association between lead and micronutrient status, and children's sleep, classroom behavior, and activity. Arch Environ Occup Health 62:105–112

Lakshmi Priya M, Geetha A (2010) Level of trace elements (copper, zinc, magnesium and selenium) and toxic elements (lead and mercury) in the hair and nail of children with autism. Biol Trace Elem Res 142:148–158

Markowitz M (2000) Lead poisoning. Pediatr Rev 21:327–335

Matte TD (2003) Effects of lead exposure on children's health (in Spanish). Salud Publica Mex 45(Suppl 2):S220–S224

Meleka LK, Ismail ME (1999) Arabic translation of Wechsler intelligence scale for children, 6th edn. Egyptian Anglo library, Cairo

Mostafa GA, Al-Ayadhi LY (2011) The possible link between the elevated serum levels of neurokinin a and anti-ribosomal P protein antibodies in children with autism. J Neuroinflammation 8:180. doi:10.1186/1742-2094-8-180

Mostafa GA, El-Sayed ZA, Abd El Aziz MM, El-Sayed MF (2008) Serum anti-myelin-associated glycoprotein antibodies in Egyptian autistic children. J Child Neurol 23:1413–1418

Mostafa GA, El-Hadidi ES, Hewedi DH, Abdou MM (2010) Oxidative stress in Egyptian children with autism: relation to autoimmunity. J Neuroimmunol 219:114–118

Petranovic D, Batinac T, Petranovic D, Ruzic A, Ruzic T (2008) Iron deficiency anaemia influences cognitive functions. Med Hypotheses 70:70–72

Piomelli S (2002) Childhood lead poisoning. Pediatr Clin N Am 49: 1285–1304

Quig D (1998) Cysteine metabolism and metal toxicity. Altern Med Rev 3:262–270

Schopler E, Reichler RJ, Renner BR (1986) The childhood autism rating scale (CARS) for diagnostic screening and classification in autism. Irvington, New York

Singh VK, Hanson J (2006) Assessment of metallothionein and antibodies to metallothionein in normal and autistic children having exposure to vaccine-derived thimerosal. Pediatr Allergy Immunol 17: 291–296

Singh VK, Rivas WH (2004) Prevalence of serum antibodies to caudate nucleus in autistic children. Neurosci Lett 355:53–56

Singh VK, Lin SX, Yang VC (1998) Serological association of measles virus and human herpesvirus-6 with brain autoantibodies in autism. Clin Immunol Immunopathol 89:105–108

Tian Y, Green PG, Stamova B, Hertz-Picciotto I, Pessah IN, Hansen R, Yang X, Gregg JP, Ashwood P, Jickling G, Van de Water J, Sharp FR (2011) Correlations of gene expression with blood lead levels in children with autism compared to typically developing controls. Neurotox Res 19:1–13

Toubi E, Shoenfeld Y (2007) Clinical and biological aspects of anti-P-ribosomal protein autoantibodies. Autoimmun Rev 6: 119–125

Vojdani A, Campbell AW, Anyanwu E, Kashanian A, Bock K, Vojdani E (2002) Antibodies to neuron-specific antigens in children with autism: possible cross-reaction with encephalitogenic proteins form milk, chlamydia pneumoniae and streptococcus group a. J Neuroimmunol 129:168–177

Vojdani A, Pangborn JB, Vojdani E, Cooper EL (2003) Infections, toxic chemicals and dietary peptides binding to lymphocyte receptors and tissue enzymes are major investigators of autoimmunity in autism. Int J Immunopathol Pharmacol 16:189–199

Wechsler D (1991) Examiner's manual: Wechsler intelligence scale for children, 3rd edn. Psychological Corporation, New York

Yorbik O, Kurt I, Hasimi A, Ozturk O (2010) Chromium, cadmium, and lead levels in urine of children with autism and typically developing controls. Biol Trace Elem Res 135:10–15

Zheng W, Aschner M, Ghersi-Egea JF (2003) Brain barrier systems: a new frontier in metal neurotoxicological research. Toxicol Appl Pharmacol 192:1–11