Case Report　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　**Open Access**

Evaluation of Blood Lead Level as a Risk Factor in Children with Autism Spectrum Disorder: A Case Control Study

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Abstract

The present study deals with the Evaluation of Blood lead (Pb) level as a risk factor in Autistic Children and to determine the association between blood lead level (BLL) and Autism Spectrum Disorder (ASD). It was a case-control study. Blood samples were collected from both case (25 of 3-16 years) and control (25 of 3-16 years) groups by vein puncture for the determination of blood lead levels using Energy Dispersive X-ray Fluorescence (EDXRF) technique. Predesigned questionnaires were completed for each case and control group by interviewing the parents or care-givers. The present study revealed that there was a significant difference between mean ages of mother at child's birth in both case and control group. Significantly more children in case group had parents with higher educational levels and came from families with higher socioeconomic status. Significantly more children in ASD group came from urban areas than rural area. The risk of exposure to air pollution in case group was 14 times more than the control group which is represented by the proximity of child's residence to high traffic roads. History of pica was exclusively present in case group (p value 0.001) indicating that children in ASD group had more exposure to lead than those of control group. The mean blood levels were 44.18 and 29.22 µg/dl for case and control group respectively. In case group 48% of the children had blood lead level ≥ 10 µg/dl compared to 24% in the control group.

Keywords: Autistic; Blood lead level; Case group; Control group; EDXRF

Introduction

Autism is a devastating childhood condition that has emerged as an increasing social concern. It has increased in prevalence in recent decades. Environmental, genetic and epigenetic factors all play a role in determining the risk of autism. Toxic levels of heavy metals have been suggested to play a critical role in the pathogenesis of autism spectrum disorder (ASD). Several studies have evaluated associations between autism and biomarkers of heavy metal exposure and effects. The evidence that heavy metal exposure leads to an autistic phenotype is increasingly convincing. Sulfhydryl-reactive metals including arsenic, cadmium, lead, and mercury are the metals most commonly reported as being associated with autism prevalence and risk [1].

An emerging hypothesis states that autism may result from a combination of genetic susceptibility and exposure to environmental toxins at critical periods during brain development [2]. Several studies have explored the levels of heavy metals and essential minerals among children diagnosed with ASD [3]. Johnson & Myers [4] suggested that environmental exposures may act as central nervous system teratogens in early gestational life. Among the toxic heavy metals lead is a pervasive environmental contaminant that can be ingested from various sources, including air pollution, lead paint and house dust contaminated by lead paint, as well as soil, drinking water and food.

Studies have the developing fetus and children are more sensitive to lead exposure than adults because of the immaturity of the blood-brain barrier, increased gastrointestinal absorption and hand-to-mouth behaviors. Several suggested the possible association between exposure to lead and ASD. Hence lead (Pb) is a toxic metal shown to cause neuro developmental disorders in children [5]. Several studies have investigated the possible association between exposure to lead and ASD, but their findings are conflicting [6,7]. Adams et al. [8] conducted a study where they involved 55 children with autism ages 5-16 years compared to 44 controls of similar age and gender and reported that the autism group had significantly higher levels of lead in their red blood cells and higher urinary levels of lead, thallium, tin, and tungsten. Lead, thallium, tin, and tungsten are toxic metals that can impair brain development and function, and also interfere with the normal functioning of other body organs and systems.

Therefore, in a country like Bangladesh where there have been recent rapid urbanization and subsequent massive exposure of the children to numerous environmental pollution, it is important to find out an association between blood lead concentration and autism spectrum disorder (ASD). Limited data is available in our country evaluating blood lead level and ASD and hence present study was conducted to determine the blood lead level of children with ASD and that of controls and to compare the demographic characteristics of the patients with ASD & that of controls. It was also elucidating whether lead is a risk factor in ASD or not.

Materials and Methods

Study population

This is a case control study, carried out during the time period of July 2015 to June 2016. A total of 50 children were enrolled in this study. Among them 25 were diagnosed cases (Children of 3-16 years of age) of autism spectrum disorder (ASD) presenting to OPD, IPNA for follow up and 25 children (Children of 3-16 years of age) who presented to OPD & got admitted to inpatient department of Pediatrics, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. Purposive sampling method was used in this study.

Sample size

Sample size was determined using sample size for hypothesis testing of the difference between two means (Equation 1) Hoque, et al. [9].

$$n = \frac{(Z\alpha + Z\beta)^2 \times (\Omega 1^2 + \Omega 2^2)}{(\mu 1 - \mu 2)^2} \quad \ldots\ldots\ldots\ldots (1)$$

where,

n= sample size for each group

μ1= Mean of one group (Case group); Ω1= SD of one group (Case group): from previous study.

μ2= Mean of other group (Control group); Ω2= SD of other group (Control group): from previous study.

Zα= Z-value of standard normal distribution (SND) at given level of significance.

Zβ= Z-value of SND at a given power.

Zα= Tow tailed Z- value of standard normal distribution at 95% confidence level which is 1.96.

Zβ= Two tailed Z- value of standard normal distribution at power of 0.80(Type-II error=0.20) which is 0.84.

Putting the values from previous study Rahbar et al. [5] in the equation (1) the sample size 'n' was estimated as:

$$n = \frac{(1.96+0.84)^2 \times (2.04^2 + 1.98^2)}{(3.84 - 2.31)^2}$$

=27

Therefore, 25 children were taken in each group, leading to total sample size of 50 children (Cases=25, Controls=25).

Ethical consideration

Ethical clearance was taken from Institutional Review Board (IBR) of BSMMU. The parents were informed about the study design and its objectives. They were explained that there would be no physical or social risk for the participants. They were also informed about freedom to participate or not to participate at any time. No incentive was given for participation. According to a predesigned questionnaires the demographic and socioeconomic data were collected from both case and control group.

Data collection procedure

The cases (ASD group) were collected from the outpatient department (OPD) of Institute of Pediatric Neuro disorder and Autism (IPNA), BSMMU who were previously diagnosed as having Autism Spectrum Disorder based (ASD) on the basis of 'Diagnostic and statistical manual of mental disorders, fourth edition, text revision (DSM-IV TR)' criteria. The controls were collected mainly from outpatient departments of Pediatrics and few were collected from inpatient department of Pediatrics who was admitted for conditions other than autism spectrum disorder (ASD) and who was not in serious conditions. Predesigned questionnaire were completed for each case and control by interviewing the parents or care-givers. Data were collected regarding demographic and socioeconomic status (SES) of the participants, information about parental educational levels. Information were collected about the children's potential exposure to lead through conditions of the households where the children were living in, through proximity of the child's home to a high traffic roads (whether or not the child's home was within a quarter of a mile of a high traffic road), and whether the child lived in a home that was located within a mile of automobile battery repair factory, automobile battery recycling centers, or battery processing facilities, through absence or presence of abnormal food habit, i.e., pica, sources of drinking water and through H/O passive smoking of mothers during pregnancy and childhood.

Collection and preparation of blood sample

Blood samples were collected in numerically labeled plastic containers. Before collecting blood 50 plastic containers were pretreated in Atmospheric and Environmental Chemistry Laboratory of Atomic Energy Center, Dhaka. Containers were kept in 20% Nitric acid for 24 hours. Then all were washed with tap water several times and finally rinsed with deionized water and dried. The empty weight of all containers were taken and kept in a plastic covering. Five ml of blood from each case & control group was taken and kept in -20°C in ultra-freezer in the Department of Biochemistry, BSSMU before transportation to the subsequent laboratory. Aseptic measures were followed strictly during collecting blood samples .The samples were dried in an oven at 60°C-70°C until constant weight was obtained. The dried mass were powdered in a carbide mortar with the help of a pestle and preserved in a desiccator until subsequent analysis. For the preparation of pellet 0.1 g of each of powdered sample was pressed into a pellet of 0.7 cm diameter and 1 mm thickness using a pellet maker (CARVER, 10 mm, model no:018735C) using 3 ton pressure. All samples were analyzed for lead (Pb) concentration using X-ray Fluorescent (XRF) Spectrometry.

Sample irradiation

The sample pellet was irradiated with 30 mCi Cd-109 radioisotope annular source for about 1000 seconds to excite the characteristic X-rays of the elements present in the sample. The X-rays were detected with the Si(Li) detector of 170 eV resolution. The X-ray spectrum of each sample was collected by a multichannel analyzer and transferred to a computer for storage, processing and evaluation of the net X-ray intensities. Thus the concentration of blood lead was measured from the sample [10,11]. The method validation was carried out by constructing a calibration curve using commercially available standard material (Apple Leaf/NIST 1516, Spinach/NIST 1570a, Tomato Leaf/NIST 1573a, Peach Leaf/NIST 1574) and checking the relevant elemental concentration trough analysis of a standard reference

material (Orchard Leaf/NIST 1571). The whole method was described elsewhere [12].

Statistical analysis

The obtained data were checked, verified and analyzed by Statistical Package for Social Sciences (SPSS, version 22). Data were expressed as frequency, percentage, range, mean, median and standard deviation (±SD) [13,14]. Mann-Whitney U test, unpaired student's t-test, Chi-square test, Fisher's exact test were performed to evaluate the association between the variables and a p-value ≤ 0.05 was accepted as level of significance at a 95% confidence interval (95% CI).

Result and Discussion

Analysis of demographic and socioeconomic data

Distribution of age and sex of the study children

Among the total 50 children, the ASD group (25) had a mean age of 8.83 years, range 3-16 (14 are 3 to 10 and 11 are 10 to 16) while the mean age for children in the control group (25) was 10.80 years, range 3-16 (10 are 3 to 10 and 15 are 10 to 16) respectively. There was no statistically significant difference (p value 0.105) between ASD and control groups regarding age distribution. In case of sex, among the total 25 children in the ASD group, 80% were male and 20 % were female.

Among 25 children in the control group, 56 % was male and 44 % was female. There was no statistically significant difference (p value 0.069) between ASD and control groups regarding sex distribution. But in the ASD group the ratio between male and female was 4:1.

Effect of maternal ages and monthly family income at child's birth

The findings are showed in (Table 1) and revealed that the mean age of the mothers in case group was higher than that of control group which was statistically significant. This age distribution signifies that high maternal age is a risk factor for autism.

Table 2 shows that the majority (92%) of children in case group was from high income group. Socioeconomic status was significantly different between the two groups. This observation is consistent with the study conducted by Rahbar et al. [5] and Durkin et al. [15]. Higher socioeconomic status in children with ASD and probable explanation between the increased [16,17] incidence of autism and higher socioeconomic status (SES) might be that there may be a possibility of higher exposures of some consumer products which may act as environmental teratogens [18].

Effect of educational status of parents by groups

The educational level of the parents by the group is shown in Table 3. From Table 3 it was evident that educated families were significantly more in case group than the control group [19-23].

Maternal ages at child's birth (years)	Group		p value*
	Case	Control	
	N (%)	N (%)	
≤35	24 (96.0)	25 (100.0)	
>35	1 (4.0)	0 (.0)	
Total	25 (100.0)	25 (100.0)	
Mean ± SD (y)	27.13 ± 4.79	23.44 ± 5.44	0.014 s
*t test was done to measure the level of significance. S: Significant			

Table 1: Comparison of maternal ages at child's birth by groups (n=50).

Family income (Tk.)	Group		Odds Ratio	95% CI of Odds Ratio	p value*
	Case	Control			
	N (%)	N (%)			
>25000	23 (92)	11 (44)	14.64	2.82-75.95	0.001s
≤ 25000	2 (8)	14 (56)			
Total	25 (100)	25 (100)			
*Chi square test was done to measure the level of significance. CI: Confidence Interval. S: Significant					

Table 2: Comparison of the family income per month by groups (n=50)

Educational levels of fathers	Group		Odds Ratio	95% CI of Odds Ratio	p value*
	Case	Control			
	N (%)	N (%)			
HSC and above	20 (80)	4 (16)	21	4.92-89.56	0.001s
Up to SSC	5 (20)	21 (84)			
Total	25 (100)	25 (100)			
Education levels of mothers					
HSC and above	19 (76)	3 (12)	23.22	5.10-105.73	0.001s
Up to SSC	6 (24)	22 (88)			
Total	25 (100)	25 (100)			
*Chi square test was done to measure the level of significance. CI: Confidence Interval n: significant					

Table 3: Comparison of the Parental educational levels by groups (n=50)also found statistically different in the present study (Table 3) which is consistent with a previous study done by Hertz-Picciotto et al. [21], Meter et al. [24]and Rahbar et al. [5]. It may be assumed that parents with higher educational status belong to higher socioeconomic status that further increases the possibility of exposing the children to more environmental teratogens in the form of consumer products [25,26].

Distribution of the places of birth, residence, proximity to High traffic road of the children by groups

Observations	Group		Odds Ratio	95% CI of Odds Ratio	p value*
	Case	Control			
	N (%)	N (%)			
Place of Birth					
Urban	20 (80)	8 (32)	8.5	2.34-30.91	0.001s
Rural	5 (20)	17 (68)			
Place of Residence					
Dhaka	8 (32)	10 (40)	0.71	0.22-2.25	0.556
Outside	17 (68)	15 (60)			
Proximity to high traffic road					
Yes	23 (92)	11 (44)	14.64	2.82-75.95	0.001s
No	2 (8)	14 (56)			
Passive smoking					
Yes	10 (40.0%)	1 (4.0%)	16	1.86-137.97	0.002s
No	15 (60.0%)	24 (96.0%)			
*Chi-square test was done to measure the level of significance. CI: Confidence Interval S: Significant					

Table 4: Distribution of different variables by groups (n-50)

This observation is consistent with Mullick et al. [27] and Rahbar et al. [5]. It was observed that places of birth of the studied children followed a statistically significant distribution between ASD and control groups (Table 4) [27-30]. Most of the children in ASD group came from urban areas (80%).

Mitra et al. [30] found that blood lead levels at the urban industrial area were significantly higher than those at the rural areas. In this

study among the case group 32% of children came from Dhaka and 68 % from outside Dhaka whereas among the control group 40% of children came from Dhaka & 60% came from outside Dhaka which indicated that living in Dhaka seemed not to be a risk factor for developing autism [31,32].

Kaiser et al. [33] identified that mean blood lead level in children at five primary schools in Dhaka was above the CDC's level of concern (10 μg/dl) [34,35]. Despite reduction in airborne lead associated with the phasing out of lead-based fuels, soil near roads and freeways is still contaminated with lead dust Mendola et al. [36].

In this study a positive relationship was found between proximity of residence to freeways [37-41] and major roadways (≤ 400 meters) during pregnancy and postnatal period and ASD (Table 4) which is consistent with Volk et al. [39].

In another study Volk et al. [42] found that Children with autism were more likely to live at residences having highest exposure to traffic-related air pollution, during gestation compared with control children.

Maternal active and passive cigarette smoking is associated with lower global intelligence, impaired visuospatial functioning, and lower language [43,44] and reading scores Mendola et al. [36]. Maternal smoking has also been implicated as a risk factor for idiopathic mental retardation Drews et al. [45]. In the present study history of passive smoking during pregnancy [46,47]and postnatal period was identified in mothers of 10 (40%) & 1 (4%) children in ASD and control groups respectively (Table 4). This observation is supported by the previous studies by Fried et al. [48] and Blaurock-Busch et al. [49]. History of active smoking was found neither in ASD nor in control group which is possibly consistent with our cultural background. It is expected that future researchers will focus on conducting research with larger sample to explore the definite association between exposure to lead and autism spectrum disorder.

Distribution of home environment (House with paint peeling or chipping off), sources of drinking water, presence of pica by groups (n=50)

From Table 5, it was evident that proximity of residence to freeways and major roadways (≤ 400 meters) in case group was significant compared to control group (p-value 0.001). This observation indicates that the children in ASD group had more exposure to air pollution than those of control group.

Variables	Group		p value*
	Case	Control	
	N (%)	N (%)	
House with paint peeling or chipping off			
Yes	4 (16.0)	0 (.0)	*0.110ns
No	21 (84.0)	25 (100.0)	
Sources of drinking water			
Piped water	11 (44)	6 (24)	**0.136ns
Tube-well water	14 (56)	19 (76)	
Presence of pica			
Yes	12 (48)	0 (0)	**0.001s
No	13 (52)	25 (100)	
*Fisher's Exact test was done to measure the level of significance. **Chi-square test was done to measure the level of significance e. ns= Not significant.			

Table 5: Distribution of home environment (House with paint peeling or chipping off), sources of drinking water, presence of pica by groups (n=50)

History of living in houses with paint peeling & chipping off in ASD compared to control group (p=0.110). Cracked, peeling or chalking paint can expose children to lead exposure through contact with dust or pica [36]. Home environment was evaluated in the present study (Table 2). It was found no statistically significant difference (Table 5) between the two groups regarding home environment which is consistent with a recent study by Rahbar et al. [5]. Piped water and tube well water as sources of drinking water were evaluated in both ASD and control groups in this study (Table 5) and [50,51] hence showed no significant difference between the two groups, which is consistent with a previous study by Rahbar et al. [5]. This may be due to the fact that children are still being exposed to lead through drinking water service lines made from lead, lead solder, or plumbing materials as it is transported from its source into homes. Lead also leaches into tap water through the corrosion of plumbing materials Brown and Margolis [52]. Table 5 shows that 48% of children in case group had pica, whereas in control group no children had pica which was statistically significant. This indicates that children in ASD group had more exposure to lead than those of control group.

Children with autism are at increased risk for lead exposure and intoxication because of exploratory oral behaviors and pica Clark et al. [53]. In the present study history of pica was present in 12 (48%) children in the ASD group [54-58] and none in the control group (Table 5). Our finding of high incidence of pica was supported by the studies of Clark et al. [53] and Rahbar et al. [5]. Mishori [59] suggested

checking serum lead levels in children who engage in geophagia since dirt may contain lead.

Effect of Blood lead (Pb) level in groups

Mean blood lead levels in ASD group was 44.18 µg/dl, whereas mean blood lead levels in control group was 29.22 µg/dl [60,61].

Blood leads level of child	Group		p value*
	Case	Control	
Mean (µg/dl)	44.18	29.22	0.158 ns
Range (µg/dl)	1.20-177.00	1.20-192.00	
Median	1.2	1.2	
Mean Rank	28	23	
n	25	25	
*Mann-Whitney U test was done to measure the level of significance. NS: Not significant.			

Table 6: Comparison of mean blood lead levels in case and control groups (n=50).

In the present study mean blood lead level in the ASD group was higher than the control group, though statistically not significant (Table 6). This observation is consistent with several other studies - Fido and Al-Saad [62], Blaurock-Busch et al. [49], Priya and Geetha [63], Al-Farsi et al. [3], El-Ansary et al. [64] and Alabdali et al. [2]. Tian et al. [27] found no significant differences in blood lead levels between case and control groups. In a study Hossain [65] found that heavy metal exposure had significant impact on increased risk of autism. Furthermore, in the present study it was found that the mean blood lead level in both case and control groups was above the threshold level (10 µg/dl) suggested by Centers for Disease Control and Prevention's (CDC). In the current study there was no significant difference was found when blood lead level above the CDC's level of concern (10 µg/dl) was compared between case and control groups (Table 6) which is in contrast with the study by Tian et al. [66] where no blood lead level was found higher than 10 µg/dl in any subject. On the other hand, Kern et al. [67], Yorbik et al. [68] and Rahbar et al. [5] reported lower levels of lead in children with ASD compared with typically developing (TD) controls.

Conclusion

In the search for pieces to the autism puzzle, a growing number of people in the autism community have turned their attention to the possible role of heavy metals specially Lead (Pb). Gene-environment interaction has long been considered a likely mechanism contributing to the risk for autism spectrum disorder (ASD). Some symptoms and behaviors observed in Autism Spectrum Disorders appear to parallel those seen in heavy metal (Pb) toxicity, eg. in chronic lead poisoning which include loss of appetite, apathy, irritability, refusal to play, constipation, decreased intelligence, hyperactivity, aggression, poor impulse control, poor eye-hand coordination, and impaired motor skill development.

Children come in contact with toxic heavy metals through inhalation, ingestion and absorption. Exposure may occur through air, water, food, or consumer products, e.g. tap water which may leach lead from pipes and solder old paint chips- particularly for children with

Difference in blood lead levels between two groups was not statistically different, though both groups have mean blood lead levels above the CDC's level of concern (10 µg/dl) (Table 6).

pica; candles with lead core wicks; leaded gasoline & poorly monitored lead emissions *via* industrial waste disposal, antique pewter and ceramic wares, pesticides and insecticides, disposed batteries, pottery, and cans. As children of Bangladesh are exposed to different sources of lead poisoning mentioned above, it is reasonable to identify whether or not elevated blood lead level is a risk factor for Autism Spectrum Disorder.

In the present study, the mean blood lead level in the case (ASD) group was higher than the control group and hence 48% children belong to case group had Blood lead (Pb) level ≥ 10 µg/dl, whereas in control group it is 24%. Significantly more children in ASD group came from families with higher socio-economic status and urban areas than rural areas which reflects more exposure to Lead (Pb) in the form of consumer products. It was also found the risk of exposure to air pollution in case group is 14 times more than the control group. Pb is an airborne particle thus more exposure to air pollution leads children more prone to lead (Pb) contamination. As children with ASD may have a poor heavy-metal-detoxifying mechanism and cannot excrete lead from their bodies compared to healthy children, elevated blood lead level seems a risk factor for the development of autism spectrum disorder.

The mean blood lead level was higher in both groups than the recommended level by the CDC which emphasizes the magnitude of the exposure to lead and thus placing children of our country at risk of abnormal neurodevelopment. Several risk factors were found which might be associated with ASD e.g. the children from families with higher socioeconomic status, parents with higher educational levels and age of parents, living in urban areas, proximity of child's residence to high traffic roads, history of pica, exposure to passive smoking in mothers during pregnancy and postnatal period, which are also found in the children with high blood lead (Pb) level. From this point of view it is quite evident that high lead level is a risk factor for Autism Spectrum Disorder (ASD). It can be suggested that to identify the definite association between blood lead level and autism spectrum disorder, further study with larger sample size should be carried out. The mean blood lead levels in both the studied groups were higher

than the CDC's recommended level of <10 µg/dl that reflects the high environmental exposures to lead poisoning in our country. Therefore, universal screening of infants and young children for lead exposure should be initiated.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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