Lead among children with autism in Iraq. Is it a potential factor?

Lead level in Iraqi children with autism

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Abstract

Aim: Autism is a neurodevelopmental disorder which affects communication and social interaction of children. It is a heterogeneous disease with various clinical presentations. Some genes are involved in its pathogenesis. It has been suggested that environmental exposure to lead can increase the risk of autism. The aim of our study was to compare blood lead levels among autistic and non-autistic children. Material and Method: This retrospective study included 107 children (60 with autism and 47 without autism) referred from the different Iraqi provinces, in the years 2015, 2016 and 2017, to the poisoning consultation center in Baghdad. Data collection including age, gender, residence, referral source, family history and blood lead levels was taken from their medical records. Results: No significant differences were noticed between the prevalence of autism and both of age (P=0.843) and gender (P=0.699). The majority of children have no family history of autism (85%). Mean blood lead level for all participants was 16.01 µg/dl. The statistically significant difference in lead level was found between autistic and non-autistic children (17.38± 1.86 µg/dl in autism group versus 14.27± 1.57 µg/dl in non-autism group) with a P value of 0.001. Discussion: Mean blood lead level was high in all participants, compared to the international values, which might indicate a high level of lead exposure in Iraqi children due to environmental pollution. Autistic children have higher lead levels than non-autistic ones and this might explain the role of lead in the etiology of autism.

Keywords

Autism Spectrum Disorder; Environment; Lead

Introduction

Awareness of autism has been expanded remarkably in the recent years [1]. Autism is not considered as a single disease, due to its wide spectrum of clinical manifestations, so the term autism spectrum disorder (ASD) has been introduced to refer to autism [2]. Autistic children have brain developmental abnormalities thus they fail to communicate or adhere with other children and they perform rigid, repetitive, and stereotypic behaviors. These neurodevelopmental disorders are classified in the Diagnostic and Statistical Manual of Mental Disorders [3]. Autism could be diagnosed prior to the age of 3 years since its symptoms can begin earlier [4].

The prevalence of ASD, among children aged 8 years, in 14 Autism and Developmental Disabilities Monitoring (ADDM) sites in the US was higher than 1% with male to female ratio of 4:1 [5, 6].

Research showed that genetic and environmental factors are involved in the pathogenesis of ASD. Mutations of mecp2, polymorphisms of CYP27B1 (important for normal vitamin D metabolism) have been linked with ASD [7]. Since 2011, studies found about 12000 genes were differentially expressed between autistic children and control group. Those studies mainly conducted on genes of brain tissue, lymphoblastoid cell lines, and gastrointestinal tissue and many genes were shown to be dysregulated in children with autism [8].

Evidence showed a possible association between mitochondrial DNA mutation and risk of ASD [9].

In the murine model, it has been found that maternal hypertension can alter the expression of genes related to ASD in the cerebellum of offspring and this could have possible role in the etiology of autism [10].

Current research shows possible increase in ASD risk with advanced parental age, trauma and hypoxia during delivery, gestational diabetes, maternal obesity and caesarian section [11].

Environmental exposure to heavy metals can negatively affect the development of child's brain. Evidence showed that children with ASD have higher levels of lead in their blood, hair, and nails [12, 13]. Children are more susceptible to lead poisoning than adults due to hand- to- mouth activities, and contact with dust, which may contain lead particles, when crawling or playing [14]. Some studies even found a correlation between lead levels and severity of autism symptoms in children [15].

Blood lead level of 10 μg per deciliter or higher was considered as the level of concern [16]. However, more recently, Centers for Disease Control (CDC) stated that blood lead level of >5 μg per deciliter in children can be associated with neurodevelopmental abnormalities [17].

Etiology of autism is still under research. Whether environmental factors play a significant role in autism is still a controversial issue. A relatively small number of studies done on Iraqi children to address the possible effect of heavy metals exposure on autism incidence.

The aim of our study was to compare lead levels among autistic and non-autistic children referred from different Iraqi provinces to the poisoning consultation center in Baghdad.

Material and Method

This retrospective study was done at the Poisoning Consultation Center in Baghdad in the period between December 2017 and May 2018. The ethical committees both of the center and of Baghdad College of medicine agreed on the strategy of the research and the research is in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consents were taken from children's parents.

In total 107 participants were involved in this study. Sixty of them were diagnosed as having ASD (case group) and 47 were non-autistic children (control group). Those children referred from different provinces in Iraq, in the years 2015, 2016 and 2017, to the Poisoning Consultation Center in Baghdad for measurement of whole blood lead levels.

The source of referral included outpatients, hospitals and private clinics by pediatricians. The autistic group was referred, to detect blood lead levels, due to behavioral abnormalities, whereas non- autistic participants were referred, to measure heavy metals levels including their blood lead levels, due to gastrointestinal symptoms.

Patients' data namely age, gender, residence, the source of referral, family history of autism and blood lead levels, were taken from their medical records.

Blood lead measurement was done at the Poisoning Consultation Center by the following method

Five ml of blood was shacked on an electrical Kahn shaker for 60 minutes. Blood was then mixed with 5 ml of 10% of trichloroacetic acid (TCA) by using a wooden stick. To complete the precipitation of the cell and protein content of the specimen, it was left in upright position for 15 minutes. The sample was centrifuged at 4500 rpm for 15 minutes. By adjustable micropipettes, the supernatant was taken and put in a dry plastic plain tube to measure lead by atomic absorption spectrometry device. The standardized procedure was used to make working standard 0, 5, 10, 15, and 20 mg/dl. The calibration curve was done. Samples, controls, and standard were directly aspirated into air- acetylene flame where the lead hollow cathode lamp was used at a wave length of 283.2 nm [18].

Statistical analysis

Statistical analysis was done by using SPSS version 20.0. Continuous variables were expressed as mean ± Standard deviation (SD). An unpaired t- test was used where appropriate. Chi-square test was also used in this study. A P value of less than 0.05 was considered significant.

Results

The total number of children included in this study was 107. Sixty children were with autism and 47 were without autism. The age of the children involved in this study is ranged from three months to thirteen years with a mean of 52.44 months and a standard deviation (SD) of ± 2.99 months. The highest proportion of study patients was found in the age group of 1 – 4 years with a percentage of 58.9 %.

The residence of the majority of children, included in this study, was Baghdad province (84 %). The distribution of study subjects by age and residency is shown in Table 1.

Table 1: Distribution of study subjects by age, residency, family history and source of referral

Variable	(Number=107)	Percentage (%)
Age (Years)		
< 1	5	4.7
1 – 4	63	58.9
5 – 9	29	27.1
≥ 10	10	9.3
Residence	n= 106	
Baghdad	89	84.0
Outside Baghdad	17	16.0
Family history of autism	n=60	
Positive	9	15.0
Negative	51	85.0
Source of referral of patients	n=105	
Outpatients	26	24.8
Private clinic	39	37.1
Hospital	40	38.1

Eighty-five percent of autistic patients had no family history of autism. The highest proportion of the study patients were referred either from hospital or from a private clinic (38.1% and 37.1% respectively). There was a missing data as the residence of one of the children and the source of referral of 2 children could not be found in their records.

Regarding the gender of the involved children, 74.8 % was male and 25.2 % was female with male to female ratio of 2.96:1 (Figure 1).
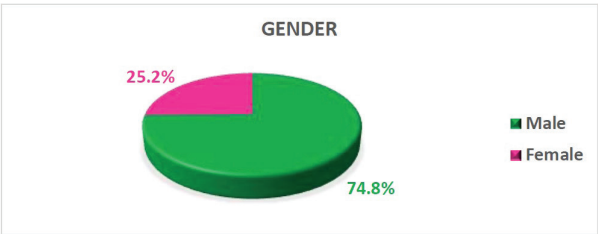

Figure 1: Distribution of study patients by gender

No significant differences were found between the prevalence of autism and both of age (P= 0.843) and gender (P= 0.699) as shown in Table 2.

Table 2: Age and sex distribution of autism

Study group	Study Groups		P – value
	Autistic children n= 60	Non autistic children n= 47	
Age group (Years)			
< 1	2 (40.0)	3 (60.0)	
1 – 4	37 (58.7)	26 (41.3)	0.843
5 – 9	16 (55.2)	13 (44.8)	
≥ 10	5 (50.0)	5 (50.0)	
Gender			
Male	44 (55.0)	36 (45.0)	0.699
Female	16 (59.3)	11 (40.7)	

The mean blood lead level was found to be 16.01 µg/dl for all participants, 17.38 µg/dl for autistic patients (the highest among study groups) and 14.27 µg/dl for the non- autistic group (Figure 2).

Comparing blood lead levels between autistic and non-autistic children showed that the blood lead level among patients with autism was higher than that among non- autistic group (17.38 ± 1.86 versus 14.27 ± 1.57) and this difference was statistically significant (P=0.001) (Table 3).
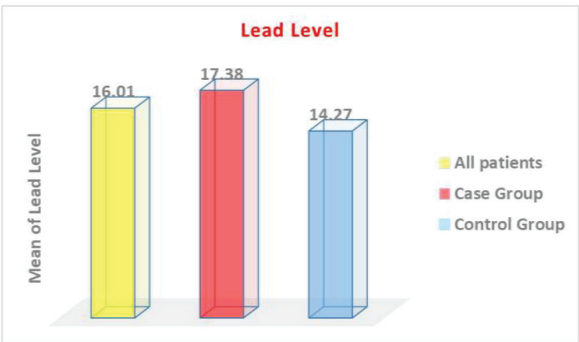

Figure 2: Mean of blood lead level among study groups

Table 3: Comparison of lead level between the study groups

Lead Level	Lead level in Study Groups		
	Children with autism Mean ± SD	Children with no autism Mean ± SD	P-Value
	17.38 ± 1.86	14.27 ± 1.57	0.001

Discussion

This study involved 107 children referred from different Iraqi provinces to the Poisoning Consultation Center in Baghdad. Sixty of them were diagnosed with autism and referred for measurement of blood lead level at this center. Forty seven children were referred for the measurement of metals levels including blood lead level.

Regarding family history of autism, studies observed that positive family history of autism increases the risk of having ASD suggesting the role of genetics in autism etiology [19, 20]. However, in this study, 85 % of autistic children were found to have negative family history, which may be attributed to the relatively small sample size.

Most of the autistic children included in this study were between 1 and 4 years old which goes in correspondence to what Mandell et al. found [21]. However, in this study the association between the prevalence of autism and different age groups was not significant (P= 0.843). The age of diagnosis depends on many factors as more severe symptoms, higher parents social, economic, and educational levels, are associated with early detection [22].

Most studies suggested that males have more susceptibility to be affected by ASD than females [5, 6]. However, the opposite was found in ASD in Smith-Magenis syndrome as female to male ratio was higher [23]. In this study, there were no statistically significant differences between the prevalence of autism and gender, this result is in agreement with Postorino and her team [24] as male bias could be explained by presence of female protecting factors and not due to male related risk factors. Nonetheless, molecular explanations are needed for full understanding of inconsistent results between the studies regarding gender effect on autism prevalence [25].

In this study, blood lead level was found to be high for all participants attended the Poisoning Consultation Center with a mean of 16.01 µg/dl and this level is considered very high compared to the international blood lead levels as blood lead level of >5 µg/dl can be considered a cause of neurodevelopmental abnormalities [17].

This high level of lead in Iraqi children may be due to their exposure to heavy metals such as lead that polluted the environment as observed by an Iraqi study [26]. Diesel and leaded gasoline are still be used as fuel in Iraq. Also, more recently, the effect of increasing transportation ways including private and public transports and the usage of local generators have increased environmental pollution in Iraq [27]. Whereas, in developed countries, such as the US, banning of the usage of leaded gasoline and the decrease of smelters smokestack emissions have reduced the median of blood lead level in children aged 1-5 years, from 15 µg/dl in the years between 1976 and 1980 to 1.9 µg/dl in the year 1999 [28].

Our results showed that autistic children have significantly higher blood lead level than non-autistic children with means of 17.38 µg/dl and 14.27 µg/dl respectively. Despite the genetic contribution to autism etiology, suggested by finding many genes with variable expression in ASD [7, 8], evidence have shown participation of environmental factors, such as lead exposure, in the etiology of autism which agreed with our study results [12, 13]. However, some studies could not observe the same finding regarding lead exposure and autism [29].

Environmental factors might influence autism by causing oxidative stress (free radicals) and impairment of methylation in autistic children and that may be due to the effect of toxic metals on sulfur metabolism [30]. In addition to lead exposure in the environment, high blood lead level in children with ASD could result from defect in lead metabolism in those children [12].

Since there is no threshold of lead negative impact on health [31], it is important to lower lead level in the environment as much as possible in order to reduce having autism (resulted from environmental exposure) in children with genetic susceptibility to this disease. The involvement of lead in autism etiology can be prevented thus minimize the effect of lead exposure on autism prevalence [32].

The main strength of our study is that our participants were referred to the Poisoning Consultation Center and that may reflect the lead levels in Iraqi children as it receives referral from different Iraqi provinces. A relatively small sample size and being a retrospective study can be considered as weaknesses.

It is essential to do further research with a larger sample size to shed more light on the etiology of ASD.

Conclusion

Etiology of autism is complex and it involves environmental factors in addition to genetic background as lead level was significantly higher in autistic children in this study. In Iraq, the environment is polluted by heavy metals since the mean level of blood lead in all participants was high compared to international levels. So prevention methods and presence of guidelines and educational programs are important in reducing autism prevalence by minimizing the effects of lead exposure.

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Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study 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. No animal or human studies were carried out by the authors for this article.

Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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