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Original article

Study of serum copper and ceruloplasmin levels in Egyptian autistic children

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ABSTRACT

Background: Autism is a behaviorally defined neurodevelopmental disorder of unknown etiology.
Objective: To assess serum copper and ceruloplasmin levels in Egyptian autistic children patients.
Subjects and methods: 40 participants have been subjected to thorough history taking, complete clinical examination, IQ assessment, estimation of serum copper and ceruloplasmin levels.
Results: A statistically significant difference was found between patients and controls as regards stereotypic movements, absent eye contact, delayed motor development, delayed speech and IQ ($p < 0.01$ for each item). Mean level of copper was significantly higher in patients than in controls ($P < 0.001$), also mean level of ceruloplasmin was significantly higher in patients than controls ($P = 0.009$).
Conclusion: Serum copper level may have a role in the pathogenesis of autism.

1. Introduction

Autism is a syndrome characterized by impairments in social relatedness, communication, repetitive behavior, abnormal movements and sensory dysfunction [1].

Copper, an essential trace mineral, is vitally important for both physical and mental health. Copper is critical for energy production in the cells. It is also involved in nerve conduction, connective tissue, the cardiovascular system and the immune system. Copper stimulates production of the neurotransmitters epinephrine, norepinephrine and dopamine. It is also required for monoamine oxidase, an enzyme related to serotonin production [2].

Ceruloplasmin is the major copper-carrying protein in blood, and in addition plays a role in iron metabolism. First described in 1984, ceruloplasmin carries about 70% of the total copper in human plasma while albumin carries about 15% [3].

Autistic children are frequently subjected to oxidative stress; hence the levels of major antioxidant serum proteins like ceruloplasmin (copper-binding protein) are decreased in children with autism. Heavy metals –including copper– are found at higher values in serum of autistic individuals than their normal peers [4].

2. Aim of the work

To assess serum copper and ceruloplasmin levels in Egyptian autistic children patients.

3. Subjects and methods

The present study was designed to be of a case control type. It enrolled 20 cases with autism diagnosed by ICD-10 (International Classification of disease, 10th edition) and the Childhood Autism Rating Scales (CARS), with no other medical disease. The patients were 15 males and 5 females. Their ages ranged from 2 to 13 years with mean of 6.15 ± 3.133 years. They were recruited from the psychiatric clinic of pediatric department and psychiatric department, Ain Shams University, during the period from December 2011 to April 2012.

The control group enrolled 20 apparently healthy children, matched to the patients' age and sex. They were recruited from the outpatient clinic, children's hospital, Ain Shams University. Their ages ranged from 4 to 17 years with a mean of 9.05 ± 3.605 years.

All patients were subjected to:

1. Thorough history taking laying stress on social interactions, impairment of language, age, gender, age of onset of clinical manifestation, presence or absence of perinatal problems and family history of psychiatric disorders.

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2. Complete clinical examination with special emphasis on neurological examination.
3. Psychiatric evaluation including:
   a- IQ assessment using Stanford-Binet Intelligence Scale (1986) [5].
   b- Assessment of the severity of autistic symptoms using Children Autism Rating Scale (CARS) [6].
4. Laboratory investigations including serum copper and ceruloplasmin levels [7].

3.1. IQ assessment

Using Stanford-Binet Intelligence Scale (1986) was carried out by clinical psychologists at the psychiatry department, Ain Shams University Hospitals. IQ was calculated using the following equation:

$$IQ = \frac{Mental\ age\ (MA)\ assessed\ using\ the\ proper\ test}{Chronological\ age\ (CA)} \times 100$$

Ranges of IQ: 20–30 severe mental retardation, 31–49 moderate mental retardation, 50–70, mild mental retardation, 71–89 below average IQ, 90–109 normal IQ, 110–12 above average IQ and 125–140 genius.

Items of the CARS are: relating to people, imitation, emotional response, body use, object use, adaptation to change, visual response, listening response, taste, smell and touch response and use, fear or nervousness, verbal communication, non verbal communication, activity level, level and consistency of intellectual response and general impressions.

3.2. Collection of blood samples

Specimen collection and storage: The usual precautions for puncture were observed and followed. Blood samples were collected by a syringe with a wide bore needle 5 ml. All samples were drawn from non fasting individuals. Evacuation of the sample was done after removal of the needle very slowly over the side of the test tube to avoid hemolysis without anticoagulant. Don't shake the tube to avoid hemolysis. The samples were stored at room temperature for 2 h then centrifugation was done for serum separation. Serum was then collected at eppendorf tube and frozen at −20 C for storage.

3.3. Statistical methods

IBM SPSS statistics (V. 20.0, IBM Corp., USA, 2011) was used for data analysis. Data were expressed as Mean ± SD for quantitative parametric measures in addition to both number and percentage for categorized data. Student *t* test was used to compare between two independent mean groups for parametric data, person correlation test was done to study the possible association between each two variables among each group for parametric data. Chi-square test was done to study the association between each 2 variables or comparison between 2 independent groups as regards the categorized data.

The probability of error p value = 0.05 or less was considered significant.

4. Results

A statistically highly significant difference was found between patients and controls as regards stereotypic movement, delayed motor development (90% of patients), absent eye contact (85% of patients), all our patients have abnormal speech and low IQ ($p < 0.01$). The difference between patients and control groups was statistically insignificant ($P > 0.05$) as regards convulsions (5% of patients). All patients and controls have taken M.M.R vaccine (Table 1).

There was statistically highly significant difference between mean level of copper in patients and controls ($P < 0.001$). Copper

Table 1
Statistical comparison between patients and controls as regards clinical data.

Variable		Cases n = 20		Controls n = 20		Value	P	Sig.
		No	%	No	%			
Stereotypic movements	Negative	2	10	20	100	32.727[a]	0.000	HS
	Positive	18	90	0	0			
Absent eye contact	Negative	3	15	20	100	29.565[a]	<0.01	HS
	positive	17	85	0	0			
Delayed motor development	Negative	2	10	20	100	32.727[a]	<0.001	HS
	Positive	18	90	0	0			
Normal speech	Negative	20	100	0	0	40.000[a]	<0.001	HS
	Positive	0	0	20	0			
Convulsions	Negative	19	95	20	100	1.026[a]	>0.05	IS
	Positive	1	5	0	0			
M.M.R vaccination	Negative	0	0	0	0		>0.5	NS
	Positive	20	100	20	100			

IS: insignificant. HS: High significance. NS: non significant.

Table 2
Statistical comparison between patients and controls as regards copper, ceruloplasmin levels and IQ.

Parameter	Case Mean ± SD	Control Mean ± SD	t	P	Sig.
Copper 70–140 μg/dl	132.15 ± 28.019	105.46 ± 15.607	−3.72	0.001	HS
Ceruloplasmin 20.4–40.7 mg/dl	35.175 ± 8.748	29.04 ± 4.481	−2.79	0.009	HS
IQ	52.7 ± 4.846	100.75 ± 2.468	39.51	0	HS

HS: High significance.

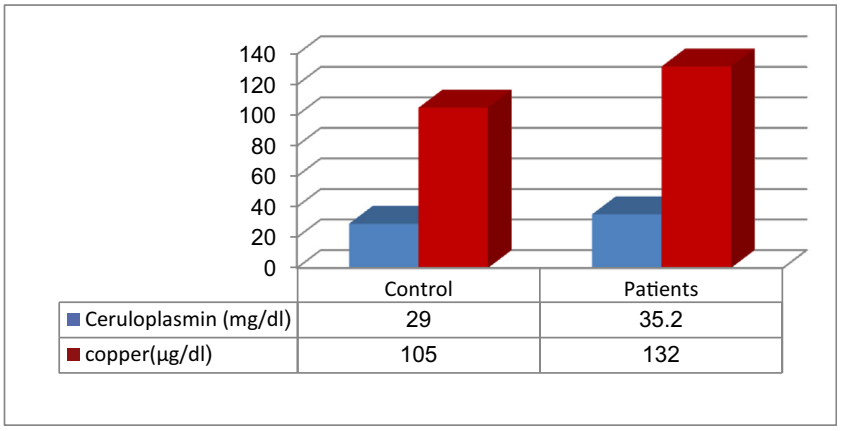

Fig. 1. Comparison between controls and patients as regards mean values of copper and ceruloplasmin.

level was higher in 30% of patients compared to normal level in controls (132.15 vs. 105.46 µg/dl). There was statistically highly significant difference between mean level of ceruloplasmin in patients and controls (P = 0.009). Ceruloplasmin level was higher in 15% of patients compared to normal level in controls (35.175 vs. 29.04 mg/dl). Using "t" test there was statistically highly significant difference between Intelligence Quotient level (IQ) in patients and controls (P<0.01) being lower in patients than controls (Table 2) (Fig. 1).

5. Discussion

Autism spectrum disorders encompass a spectrum of developmental disorders, characterized by impairment in the development of language, communication and reciprocal social interaction, together with a restricted repertoire of activities [8].

The Centers for Disease Control and prevention estimates that the current prevalence of autism spectrum disorders among 11 sites in the United States is 1 in 68 children aged 8 years [8]. Despite numerous structural and functional neuroimaging studies, as well as post-mortem investigations, the underlying neurobiological basis of autism continues to remain elusive [9]. On the other hand, awareness of the prevalence of autism in the Arab world is still very limited and no concrete reports are available from this region. The Sultanate of Oman is a country with an estimated population of 3.5 million. The prevalence estimate of ASD (Autism Spectrum Disorder) in Oman in the year 2011 was 1.4 per 10,000 children, which is comparatively low [10].

In the current study, 75% of patients were males and 25% were females, with ratio of 3:1. That is close to a study done by James et al. who found that autism affects males more than females at a ratio 4:1 [11]. According to a study done in 14 sites in USA, ASD prevalence estimates were significantly ($p < 0.01$) higher among boys than among girls in all 14 sites, with male-to-female prevalence ratios ranging from 2.7 in Utah to 7.2 in Alabama [12]. The specific factors responsible for the higher male prevalence in ASD remain unclear. ASD is not the only neurodevelopmental condition more common among males—a greater prevalence in males versus females is also seen in Attention Deficit Hyperactivity Disorder (ADHD), dyslexia; conduct disorder (CD), specific language impairment, Tourette Syndrome, and Learning Difficulties [13].

In the current study, 90% of autistic children have stereotyping which is characteristic for autism and non specific eating disorders. A study by Gal et al. stated that autistic children exceeded other groups of developmental and sensory disabilities in the frequency of 15 stereotypic movements. Also, the study stated that autistic children have self injurious behavior as a severe form of stereotyped movements [14].

In our study, all autistic children (100%) had lower IQ than controls and all in the range of 47–60. The Centers for Disease Control and Prevention reported in 2009 that the proportion of children with autistic traits who have IQs below 70 ranges from 29.3 percent in Colorado to 51.2 percent in South Carolina [15].

In our study, all our autistic children and controls have taken MMR vaccine with no difference between the two groups. This is in accordance with De Stefano who published a brief summary of several studies in the USA and proved that there is no relation of MMR vaccine with increasing autism cases [16]. Also, Melissa et al. stated that the evidence clearly shows that there is no link between vaccination against measles, mumps, and rubella, and an increased risk for the development of autism [17].

In our study, 5% of our patients had a history of convulsions. Blazek and Sansa stated that epilepsy is common in autism, with prevalence rates ranging from 7% to 46% [18]. While Kogan et al., stated that about 22–30% of children with ASD develop seizures with no specific underlying pathology, and no obvious or classic EEG changes [19]. Epileptic symptoms in children with ASD were recently considered to be related to immune-mediated pathogenesis. In fact, ASD are associated with some immune dysfunction, such as elevated antibody levels directed against the fetal brain [20].

In our study 85% of our patients have absent eye contact and all our patients (100%) have abnormal speech. This is in accordance with Klin et al., who stated that children with autism tend to focus on the area around the mouth rather than on the socially informative eye area and on static objects rather than moving people [21]. Also, Bonneh et al. stated that children with ASD who can speak often exhibit abnormal voice quality and speech prosody, but the exact nature and underlying mechanisms of these abnormalities, as well as their diagnostic power are currently unknown [22]. Hubbard and Trauner reported increased pitch range in ASD children [23].

In the current study, 90% of our patients (18 patients) have delay in motor development. This is in accordance with Esposito and Venuti who said that ASD patients showed a range of gross motor problems, including delays in motormilestones, abnormal muscle tone (velocity-dependent resistance to stretch), abnormal reflexes, and postural asymmetries [24].

Recent studies have implicated physiological and metabolic abnormalities in ASD and other psychiatric disorders, particularly immune dysregulation or inflammation, oxidative stress, mitochondrial dysfunction and environmental toxicant exposures [25].

The possible etiologies that precipitate autism symptoms remain controversial in many cases, but both genetic and environmental factors have been implicated [26]. Copper is a trace element present in all tissues and is required for cellular respiration, neurotransmitter biosynthesis, pigment formation, and connective tissue strength, and is a cofactor for numerous enzymes and plays an important role in central nervous system development. Copper may be involved in free radical production that results in mitochondrial damage, DNA breakage, and neuronal injury [27]. Copper is a component of several metalloenzymes that are linked to dopamine synthesis, in biochemical pathways involving either antagonism of dopamine production or catalysis of its breakdown. As dopamine is implicated in autism, copper homeostasis may be particularly relevant. An excess of copper may be associated with dopamine dysregulation [28].

In the current study, copper is statistically higher in patients than controls ($P < 0.001$). Similarly Faber S et al. reported high levels of serum copper in young children with autism [29]. Also a study by Russo and Robert stated that autistic children have significantly elevated plasma levels of copper ($P = 0.0133$) [30].

As well Elsheshtawy E et al. stated that the level of copper was significantly higher in cases than in controls. They also stated that the level of copper in hair was significantly associated with higher CARS scores [26]. Also in another study by Lakshmi Priya MD and Geetha A higher levels of copper was found in these children when compared with controls. Their study also concluded that the level of copper could be associated with the children's degree of autism severity [31].

Ceruloplasmin is a ferroxidase enzyme and is the major copper-carrying protein in the blood. It is an enzyme synthesized in the liver containing 6 atoms of copper in its structure. The antioxidant effects of ceruloplasmin could have important implications for various neurodegenerative diseases such as Parkinson's disease, Autism and Alzheimer's disease [4]. In our study, there was statistically high difference between mean level of ceruloplasmin in patients and controls ($P = 0.009$). On the other hands Chauhan et al. reported lower levels of ceruloplasmin in 68% of children with autism as compared to their developmentally normal siblings in the USA [32]. This difference can be explained by the fact that ceruloplasmin is an acute phase reactant that can be elevated in common infections.

6. Conclusion

Serum copper level is high among autistic children in our study, so measurement of serum copper is useful in patients with autism.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

Informed consent was taken from the parents of all children. Also approval of Ethical committee of Ain Shams University was taken . The work has been carried out in accordance with The Code of Ethics of The World Medical Association (Declaration of Helsinki) for experiments in humans.

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