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Research Article

Study of Serum Level of Serotonin in Children with Autism Spectrum Disorder

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Abstract

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by a deficit in social communications, nonverbal interactions and restricted, repetitive patterns of behavior during early developmental period. Autism is now considered an epidemic due to its rapid increase in incidence. This is why there is an intensive search to identify biological markers in genetics, neuro-imaging and measures of the body chemistry in order to achieve earlier diagnosis. ASD diagnosis is currently psychometric assessment based depending on the criteria present in the Diagnostic and Statistical Manual of Mental Disorder's fifth edition (DSM-V). Serotonin influences many physiological activities in the human body, from cardiovascular, respiration, to a more centrally controlled functions such as mood, cognition and learning. In this study, we assess the serum serotonin level in autistic children and assess the reliability of serum serotonin levels as a biomarker for diagnosis of ASD.

Method: We compared the serum serotonin levels in 40 diagnosed autistic children and 40 healthy control children by the enzyme-linked immunosorbent assay (ELISA) method.

Results: Serum serotonin was significantly high in autistic children compared to control ($P < 0.001$). However, No significant statistical difference between serotonin levels and CARS (severity of ASD) or gender was found.

Conclusion: Our study demonstrated a significantly increased serum serotonin levels in autistic children Hence, serum serotonin could be used as a biomarker for ASD diagnosis.

Keywords: *Serum; Serotonin; Autism Spectrum Disorder*

Introduction

Autism Spectrum Disorder (ASD) is a common neurodevelopmental disorder characterized by persistent impairment in reciprocal social communication and social interaction, and restricted, repetitive patterns of behavior, interests, or activities. These symptoms are present from the early developmental period, and limit or impair everyday functioning [1]. In 2016, ASD prevalence in USA was 1 in every 68 child, which is a 30% increase from that in 2008 which was 1 in every 88 child [2]. Regular screening of infants and toddlers for symptoms and signs of ASD is crucial because it allows for early referral of patients for further evaluation and treatment [3].

ASD individuals have no characteristic facial features. They also vary enormously in clinical presentation, severity and developmental trajectory. Furthermore, there is no reliable laboratory test available to assist clinicians to diagnose ASD [4]. Hence, the diagnosis of ASD is currently psychometric assessment based depending on the criteria present in the DSM-V. This complexity is spurring an intensive search

to identify biological markers able to achieve earlier diagnosis. Biological markers are also needed in predicting clinical prognosis as well as treatment response [5].

Serotonin influences many physiological activities in the human body, from cardiovascular, respiration, gastrointestinal system regulation, pain sensitivity, and thermoregulation to a more centrally controlled functions such as mood, cognition, learning, memory, the maintenance of circadian rhythm, appetite, aggression, sensorimotor activity and sexual behavior [6].

Positron emission tomography studies have demonstrated altered 5HT synthesis capacity in the autistic children's brain [7]. It showed a diminished capacity of the whole or regional brain serotonin synthesis in autistic compared to non-autistic children, thus suggesting that; developmental regulation of 5HT synthesis is involved in the pathogenesis of autism [8]. Also, Pharmacological manipulation of 5HT transmission has demonstrated an influence over some of the autistic symptoms [9].

There are two main hypotheses for the pathophysiology of ASD; the first is the blood hyperserotonin hypothesis, which has been widely accepted and confirmed. The second is the blood hyposerotonin hypothesis which has become prominent in recent years [10].

A recent study showed the similarity of platelet 5-HT to neuronal serotonin and thus the use of platelets' 5-HT activity as a peripheral marker for its central activity [11].

Aim of the Study

The aim of the present study was to assess the serum serotonin level in autistic children and assess the reliability of serum serotonin levels as a biomarker for diagnosis of ASD.

Materials and Method

Patients and participants

The present study was carried out on 40 children diagnosed with ASD, and 40 age matched healthy controls. Their ages ranged from 2 to 6 years.

The diagnosis of ASD was confirmed by the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-V) [1] and the severity was assessed by the Childhood Autism Rating Scale (CARS) [12]. All the cases included in this study were identified and selected from various medical service outlets including; the neuropsychiatric clinic in Elshatby university hospital and the child specialized center (Alexandria, Egypt).

Determination of whole blood 5-HT [13]

The serotonin assay was done using the competitive Serotonin ELISA kit (the microtiter plate format).

Statistical analysis of the data [14]

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level.

Results

Serotonin level in the two groups (Figure 1)

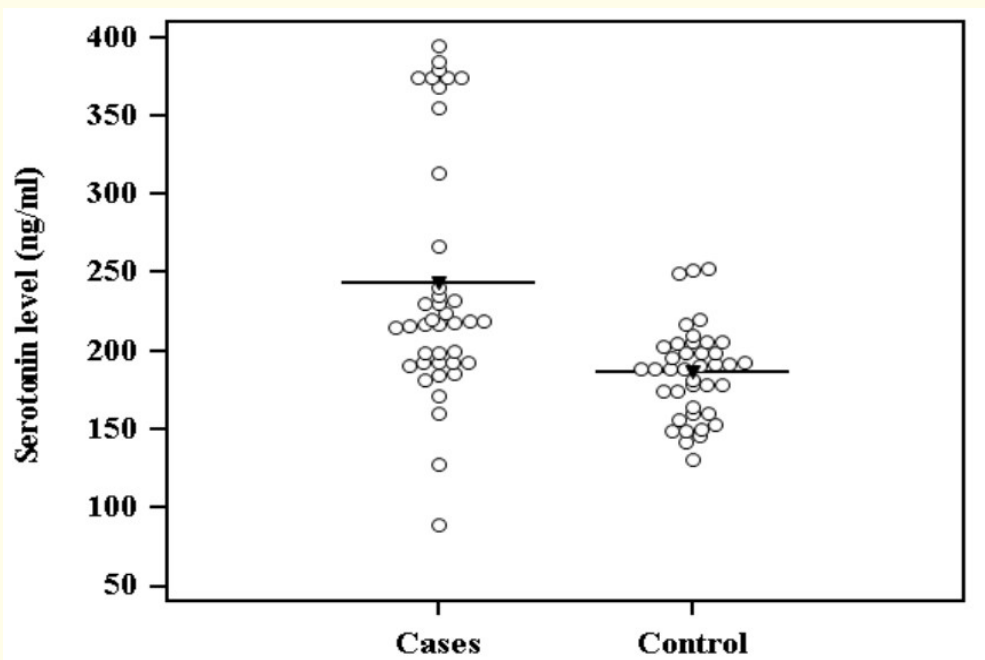

Figure 1: *Serum serotonin levels in autistic patients and healthy children. Median value for each group is shown by a horizontal bar.*

Our results should that it is statistically significant that at serotonin level > 210, there is 62.50% sensitivity and 87.50% specificity for ASD ($p < 0.001$). This curve shows that at serum serotonin level of more than 210, there is a 76% possibility that this child is autistic and justify further evaluation.

Serotonin level and gender (Figure 2, Table 1)

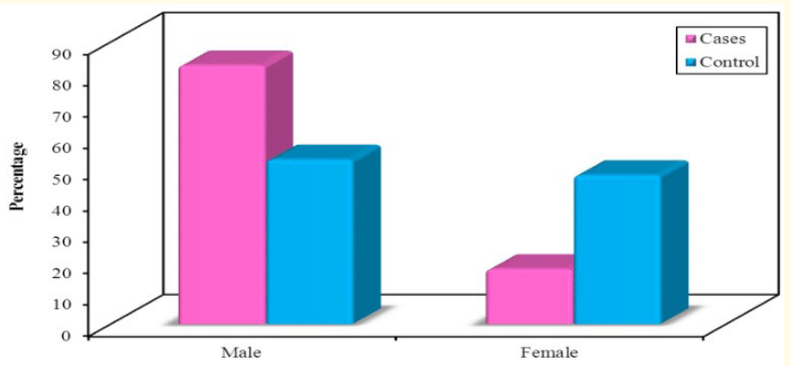

Figure 2: *Comparison between the studied groups according to gender.*

Serotonin level	Gender		t	p
	Male (n = 33)	Female (n = 7)		
Range	89.0 - 385.0	127.0 - 395.0	0.886	0.381
Mean ± SD.	248.91 ± 79.70	219.29 ± 83.78		

Table 1: *Relation between gender with serotonin level in cases group.*

In this study, there is a significant statistical difference between the male : female ratio in the two groups (P = 0.004), where the male : female ratio was 4 : 1 in the cases group while it was 1 : 1 in the control group. There is no significance statistical difference between serotonin levels in males and females, where P = 0.381.

Serotonin level and CARS scores (Figure 3, 4)

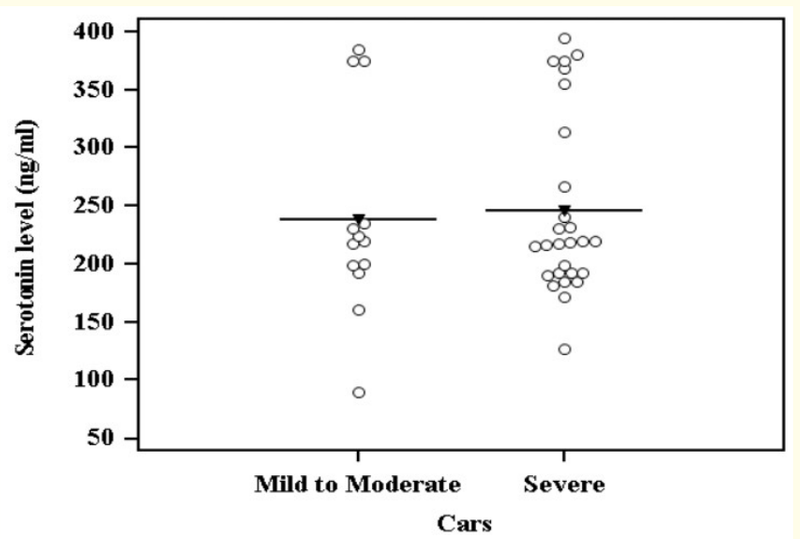

Figure 3: *Serum serotonin levels in relation to the degree of the severity of autism. Median value for each group is shown by a horizontal base.*

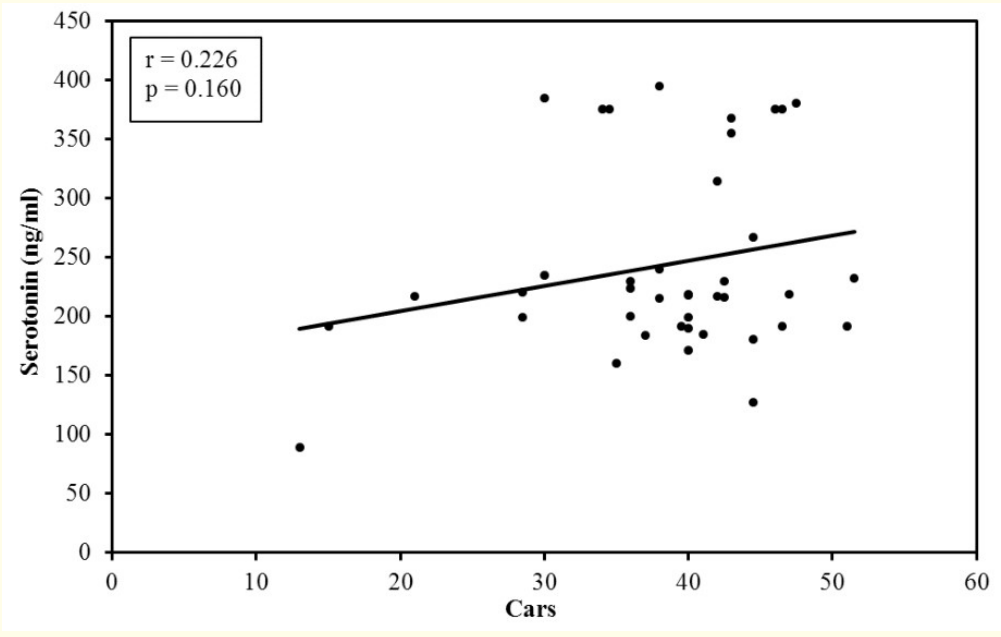

Figure 4: *Correlation between cars with serotonin (ng/ml) in cases group (n = 40).*

The present study included 13 children with severe autism (CARS 39-60) and 27 patients with mild to moderate autism (CARS 30-38). No significant difference in blood serotonin levels between the mild to moderate group (mean 238.54 ± 88.29) and the severe autistic features group (mean 246.22 ± 77.55) was found, p = 0.780.

Serotonin as a diagnostic tool for ASD (Table 2, Figure 5)

	Cutoff	Control		Cases		95% C.I		Sensitivity	Specificity	PPV	NPV
		No.	%	No.	%	LL	UL				
Serotonin level	≤ 210	35	87.5	15	37.5	0.657	0.869	62.50	87.50	83.33	70.0
	> 210	5	12.5	25	62.5						

Table 2: *Agreement (sensitivity, specificity) for serotonin level to diagnose cases from control.*

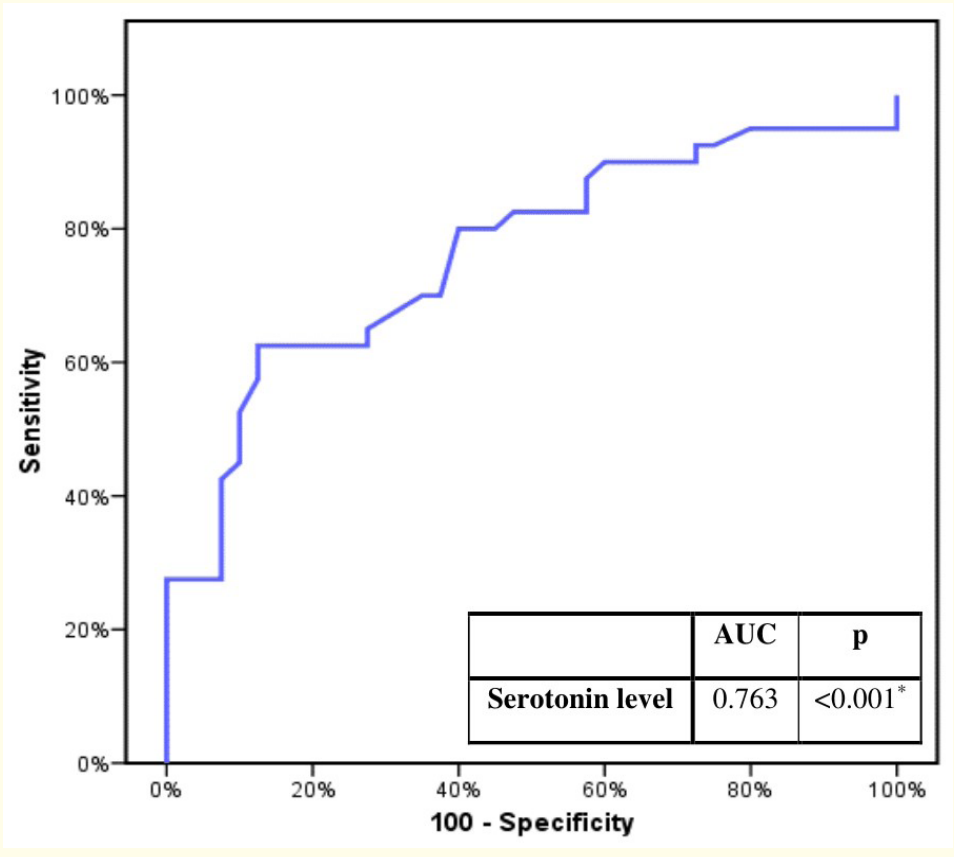

Figure 5: *ROC curve for serotonin level to diagnose cases from control.*

Our results should that it is statistically significant that at serotonin level > 210, there is 62.50% sensitivity and 87.50% specificity for ASD ($p < 0.001$). This curve shows that at serum serotonin level of more than 210, there is a 76% possibility that this child is autistic and justify further evaluation.

Discussion

Over the past 50 years much effort has been expended in the search for biomarkers for ASD. There has been a longstanding interest in the possible role of serotonin in ASD. Much of the interest can be traced back to the initial report of Schain and Freedman in 1961 [15] of elevated levels of platelet serotonin in ASD individuals. Hyperserotonemia has been defined using various different criteria including 1.5 - 2.0 standard deviations above the whole blood 5-HT mean or the 95th percentile of whole blood 5-HT in typically developing individuals [16].

In the present study, the mean values of serum serotonin levels were (186.38 ± 29.01) for control (N= 40), and (243.73 ± 80.13) for cases (N = 40). There was a significant statistical difference between serum serotonin levels in ASD individuals and healthy control ($P < 0.001$) favoring hyperserotonemia. Elevated serotonin level in either platelets, whole blood or serum was also reported by many researchers as McBride., *et al.* 1998 [17] and Gabriele., *et al*. 2014 [18]. On the other hand, Meguid., *et al*. 2015 [19] documented low platelet-poor plasma (PPP) serotonin level in autistic children. The different results could be attributed to that Meguid., *et al*. measured serotonin in platelets which is a very complicated procedure.

High 5HT level in the whole blood had been suggested to cause a low intra-cerebral 5HT concentration in ASD individuals. It is suggested that, at early stages of brain development, when the blood–brain barrier is not yet fully developed, the high levels of 5HT in the blood can enter the brain of a developing fetus and cause loss of 5HT terminals through a negative feedback mechanism. The loss of 5HT innervations persists throughout subsequent development and thus, the symptoms of autism appear [20].

Although that the cause of the increased serotonin in blood is unknown. Still a lot of theories were suggested and many controversial results were reported. For example, it had been suggested that the increased serotonin may be due to altered platelets handling of serotonin [21], increased serotonin transporter density in the platelets [22] or reduced platelet 5-HT2A receptor functioning [23].

Another theory suggested a vitamin D role. Vitamin D hormone (calcitriol) activates the transcription of the serotonin-synthesizing gene tryptophan hydroxylase 2(TPH2) in the brain at a vitamin D response element (VDRE) and represses the transcription of TPH1 in tissues outside the blood-brain barrier at a distinct VDRE. Thus, explain the increased blood serotonin levels and decreased brain serotonin levels [24].

On the other hand, recently a new hypothesis was introduced, which is the decreased blood serotonin (hyposerotonemia) hypothesis. Decreased platelet poor plasma (PPP) serotonin levels in autistic children and their mothers was reported in some studies [25]. Also, in a study where postmortem brain tissue of ASD individuals were examined, an increase in the number of serotonergic axons were observed. This situation cannot be explained by the hypothesis of compensatory mechanisms which expected to result in a feedback reduction of serotonergic axons in hyperserotonergic state [26].

Some authors also reported that serotonin and melatonin levels were significantly decreased in the blood of patients with ASD compared to healthy controls. The mechanisms underpinning this may be related to the ability of the gut microbiota to control host tryptophan metabolism along the kynurenine pathway, thereby simultaneously reducing the fraction available for serotonin synthesis and increasing the production of neuroactive metabolites [27,28].

Mulder., *et al*. 2004 [29] reported that platelet 5-HT levels were increased in PDD-NOS, while no elevation was seen in mental retardation (MR) cases. McBride., *et al*. 1998 [17] also reported that hyperserotonemia was not found in mentally retarded or otherwise cognitively impaired youngsters without autistic features. Hughes 1996 [30] reported that whole blood serotonin levels in behavior disorder, mood disorder or mixed mood did not differ significantly with the control group.

In our study, Patients with severe autism didn't have a statistically significant high serum serotonin levels [median = 232.50 ng/ml] than those with mild to moderate autism [median = 231.0 ng/ml], P < 0.291. Meguid., *et al*. in 2014 also reported that there is no significant statistical difference in blood serotonin levels between the moderate group (mean 73.11 ± SD 40.87) and the severe group (mean 85.54 ± SD 50.18).

In our study, there was no significant statistical difference between ASD male and female serotonin levels (p = 0.381). Mostafa., *et al.* [31] 2011 and Shuffrey., *et al*. 2017 [32] reported the same results, that there was no significant statistical difference between male and female serotonin levels. However, Shuffrey., *et al*. reported that males were significantly more likely to manifest hyperserotonemia than females, and this may explain the higher male prevalence in autism.

Gabriele., *et al*. 2014 in a systematic review and meta-analysis reported that they significantly reinforce the reliability of elevated 5-HT blood levels as a biomarker in ASD. In our study, we found that the optimal cut off point for using 5-HT as a biomarker for autism is 210 ng/ml, there is a 76% possibility that the child is autistic, with 62.5% sensitivity and 87.5% specificity.

Yang., *et al*. 2015 [33] using HPLC technique, reported that the optimal cut-off point for using 5-HT as a biomarker for autism was 111.20 ng/mL. This cut-off point was associated with a sensitivity of 78.79% and a specificity of 80.65%.

Conclusion

From this study we found that; ASD children have a significantly higher serotonin levels than healthy control, and can be considered as a good biomarker for early diagnosis of autism.

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