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Brain Biomarkers of some Egyptian Children with Autism manifesting poor language development

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Abstract

Autism is characterized by deficits in social interaction with restricted repetitive patterns of interests during the early developmental period. Alterations of some biochemical measures were suspected to have a role in autism pathogenesis such as dopamine, serotonin and brain-derived neurotrophic factor. These measures are important for the development of memory, language and social abilities. Hence, they could be altered in autism. We aimed to measure the blood levels of dopamine, serotonin and brain-derived neurotrophic factor in a group of Egyptian autistic children with language impairment in comparison to healthy control children to investigate the relation between them. The study included 40 children with autism and 20 healthy controls. The autism and language delay were diagnosed according to the diagnostic and statistical manual of mental disorders. The dopamine and serotonin levels for both groups were determined by high-performance liquid chromatography. The brain-derived neurotrophic factor was measured by ELISA. The level of dopamine in the autism group was less than that in the control group while the brain-derived neurotrophic factor level was higher in the autism group. Investigating the relation between dopamine and the brain-derived neurotrophic factor in the autism group revealed a statistically significant positive correlation ($r=0.5$, $p<0.01$). In conclusion, the dopamine and the brain-derived neurotrophic factor could have a role in the pathogenesis of autism. These measures could be used as diagnostic biomarkers for children suspected to have autism.

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Introduction

Autism is a mysterious disorder that leads to deficits in social interaction with the presence of restricted repetitive patterns of interests or behavior in the early period of the child's development (American Psychiatric Association, 2013). The co-occurrence between autism and intellectual disability is common. Further, the existence of a delay in language development is very frequent (Meguid *et al.,* 2018). Although the awareness towards this disorder and its prevalence is rising, establishing its diagnosis is often delayed. This delay usually hinders their progress and prevent them from getting proper management and rehabilitation in the early years of brain plasticity (Bello-Mojeed *et al.,* 2017). These children usually manifest learning difficulties, sensory processing and attention deficits. Autism has a strong genetic and environmental bases in which inflammatory markers and factors concerned with synapse formation, nerve transmission, and information processing are altered. Dopamine and serotonin are among the neurotransmitters which were suspected to be altered in autism. Dopamine is suspected to be involved in repetitive behaviors, deficits in executive functions and altered motor activities in autism (Kriete and Noelle, 2015). It is also related to immune system functions which were reported to be different in autism (Meltzer and Van de Water, 2017).

Additionally, Serotonin was implicated in the immune system, response speed control, cognitive functioning and social abilities which all are essential for language development (Schauder *et al.,* 2015). The dopamine and serotonin were reported to be influenced by the brain-derived neurotrophic factor (BDNF) (Narita *et al.,* 2003). Moreover, BDNF has a role in neuronal connectivity especially in the hippocampus and hypothalamus and peripheral sensory nerves (Duff and Brown-Schmidt, 2012). Hence, investigating the possible relations between these measures and each other and especially those with delayed language development would reveal information about the pathogenesis of autism. This study was designed to assess levels of dopamine, serotonin and BDNF in autistic Egyptian children in comparison to a sample of developmentally normal children matched age and sex. Furthermore, to investigate the association of their levels with language impairment in response to changes in an auditory stream of tones or vowels in children with autism spectrum disorders.

Patients and methods

The present study was designed to be a cross-sectional study. This study has been approved by the ethical committee of the National Research Centre and following the Helsinki Declaration. Informed consents were obtained from the parents/guardian of participants.

Patients

The study included forty children with autism spectrum disorder (group I) and twenty healthy control children (group II) with the same socioeconomic class. Group I participants were attending the learning disability research clinic in the Medical Research Centre of Excellence, National Research Centre, Cairo, Egypt.

Group I: autistic children (27 males and 13 females) with age ranged from 3.1 to 11 years (6.0 ±1.7); they were sub-grouped according to language impairment into mild language impairment (6 males, 3 females); moderate language impairment (14 males, 7 females) and marked language impairment (7 males, 3 females). Children with autism were diagnosed to have an autistic disorder and communication disorder according to the criteria of the diagnostic and statistical manual of mental disorders, 4th edition, text revision (American Psychiatric Association, 2000).

Group II: comprised healthy children serve as a control group (12 males and 8 females) with age range 3.5-9.1 (5.6. ± 1.7) with no history of motor or language delay.

The inclusion criteria were the diagnosis of autism and the presence of delay in language development. The exclusion criteria were the presence of associated

neuropsychiatric disorders, EEG changes feature suggestive of syndromic involvement, or motoric delays.

Procedures and tools

All cases were subjected to detailed history taking with special emphasis on; onset, course and duration of the disease and age, sex of the patient, consanguinity. For the assessment of children, the following assessment steps were followed:

First, mental status examination using the Standford Binet intelligence scale fifth edition (Roid, 2003) to measure the child's cognitive abilities by calculating the intelligence quotient (IQ) through assessment of 5 factors of cognition: fluid reasoning, knowledge, quantitative reasoning, visuospatial processing and working memory.

Second, preschool language scale, fourth edition (PLS-4) Arabic version (Arabic language test) (Abu-Hasseba, 2011) to pick up the syntactic profile. It is an interactive assessment of developmental language skills that can give the language age of the tested child.

Third, child autism rating scale (CARS) (Scholper *et al.,* 2010) to assess the severity of autism symptoms. CARS consists of 15 domains (relating to people; emotional response; imitation; body use; object use; listening response; fear or nervousness; verbal communication; non-verbal communication; activity level; level and reliability of intellectual response; adaptation to change; visual response; taste, smell and touch response; and general impressions). Each domain is scored on a scale ranging from one to four, with higher scores associated with a higher level of impairment. According to the scale, children who have scored 30–36 have mild autism, while those with scores ranging between 37 and 40 have moderate autism, and those with scores more than 40 have severe autism (Mick, 2005). Participants who met inclusion and exclusion criteria were screened for language impairment. Preverbal (<25 functional words) children qualified as language impaired. Otherwise, the age-appropriate version of the Clinical Evaluation of Language Fundamentals (CELF) confirmed language impairment. Language impairment was defined as a core standardized score <85 in the preschool version.

Biochemical measurement

Determination of serum dopamine and serotonin levels: They were measured by high-performance liquid-chromatography, Agilent technologies 1100 series, equipped with a quaternary pump (Quat pump, G131A model) (Hussein *et al.,* 2012). Dopamine and serotonin HPLC standards were purchased from Sigma Aldrich Chemicals Company St. Louis USA. C.

The separation was achieved on the ODS-reversed phase column (C18, 25 x 0.46 cm i.d. 5 µm). The mobile phase consisted of potassium phosphate buffer/methanol 97/3 (v/v) and was delivered at a flow rate of 1.5 ml/min. UV detection was performed at 270 nm, and the injection volume was 20 µl.

The concentration of both dopamine and serotonin were determined by the external standard method using peak areas. Serial dilutions of standards were injected, and their peak areas were determined.

A linear standard curve was constructed by plotting peak areas versus the corresponding concentrations. The concentrations in samples were obtained from the curve.

Determination of serum brain-derived neurotrophic factor: Serum BDNF concentration level was estimated by sandwich ELISA method using the Human BDNF ELISA kit (sunlong Biotech Co.) (da Costa *et al.,* 2017).

Statistical analysis

The analysis was performed using Statistical Package for the Social Science (SPSS) for Windows (version 17.0, Chicago, IL, USA). The data were analyzed by the Mann-Whitney non-parametric test. Receiver operating characteristics (ROC) analysis was

employed to find the best cutoff value of all the studied parameters. P <0.05 was considered statistically significant.

Results

A comparison between the two groups revealed that the dopamine level in autistic children was significantly lower than in the control group. The BDNF level in the autism group showed a marked increase than that in the control group with a statistically significant difference. Serotonin level did not show a significant difference between groups despite being less in the autism group (Table 1, Figs 1,2). The clinicopathological features of the autistic studied group according to language impairment was shown in Table 2.

Table 1. Comparison between the autistic and control groups regarding dopamine, serotonin and BDNF levels.

Variable	Statistics	Autistic group N=40	Control group N=20	Z	*P*-value
Dopamine (ng/ml)	Median (Q_1- Q_3)	4.6 (3.5-6.3)	6.8 (4.7-8.5)	-4.4	0.001
	Range	1.9 – 11.3	3.0 – 11.3		HS
Serotonin (ng/ml)	Median (Q_1- Q_3)	0.2 (0.2-0.3)	0.2 (0.2-0.3)	-0.02	0.901
	Range	0.1 – 0.4	0.1 – 0.4		NS
BDNF (ng/ml)	Median (Q_1- Q_3)	161 (108 –199)	73 (68 –83)	-5.3	0.001
	Range	61.0 - 270	62. - 90		HS

(Q_1- Q_3): 25th percentile- 75th percentile

Z: Mann-Whitney test value.

When comparing the levels of studied parameters among language impairment autistic subgroups, we found a statistically significant difference in BDNF level between mild, moderate and marked autistic group (, p<0.01) while serum serotonin and dopamine showed no difference (Table 3, Fig. 3).

Table 2. Clinicopathological Features of autistic children group.

Variable	Statistics	Autistic subgroups (Language impairment) N=40				
		Mild N=9	Moderate N=21	Marked N=10	Test value	*P*-value
Age (years)	Mean ± SD	6.0±1.9	6.2±2.0	5.6±1.5	F=0.4	0.82
	range	3.2 - 9.0	3.1 – 11.0	3.8 – 8.1		NS
Gender	N (%)					
Male		6 (67)	14 (67)	7 (70)	0.4	0.9
Female		3 (33)	7 (33)	3 (30)		(NS

Receiving the operating characteristics curve (ROC) was performed and it revealed that the percentage of BDNF accuracy was more than that of dopamine (Table 4, Fig. 4). A significant positive correlation was obtained between the levels of dopamine and BDNF (r=0.5, p<0.01).

Discussion

From the time of birth, a newborn is continuously exposed and naturally attracted to human voices, and as he grows, he becomes increasingly responsive to these speech stimuli, which are strong drivers for his language development and knowledge acquisition about the world. Young children with autism spectrum disorder (ASD) are often insensitive to human voices. Failure to attend to speak in turn results in altered development of language and social-communication skills (Sperdin and Schaer, 2016). Language difficulties have been reported in autism. The linguistic difficulties included semantics, pragmatics and expressive language deficits (Weismer *et al.,* 2010). Furthermore, deficits in the theory of mind and receptive language were noticed in autism (Boddaert *et al.,* 2004).

Table 3. Comparative analysis of studied parameters between different autistic subgroups based on language impairment score.

Variable	Autistic subgroups according to Language impairment Median (Q_1- Q_3) Range				
	Mild (n=9)	Moderate (n=21)	Marked (n=10)	F	P value
Dopamine (ng/ml)	4.0 (2.6 – 4.2) 1.9 – 5.2	4.0 (3.0-5.2) 2.2– 7.5	4.1 (3.1-5.0) 2.0 – 6.3	0.5	0.6 NS
Serotonin (ng/ml)	0.2(0.2 – 0.3) 0.14 – 0.3	0.3 (0.2-0.3) 0.2 – 0.4	0.2 (0.2-0.3) 0.2 – 0.4	0.9	0.4 NS
BDNF (ng/ml)	82.0 (75.0 – 89.0) * 61.0 – 97.0	160 (131 –172) ** 103 - 195	233 (205 –255) 202 - 270	91.0	0.001* HS

F: ANOVA test value;

* test is statically significant at ≤0.001 when compared to moderate and marked subgroup

** test is statically significant at ≤0.001 when compared to marked subgroup.

Studying the biochemical markers in autism is promising regarding the early diagnosis of at-risk children and regarding the potential subtyping of autism, not to mention the benefit of understanding the pathophysiological changes in such disorder ((Pavăl, 2017). Therefore, this study targeted some biomarkers that were suspected to be implicated in autism yet have not been investigated in Egyptian autistic children with investigating their role in language impairment.

Table 1. Diagnostic performance of dopamine, serotonin and BDNF in the autistic group.

Variable	Cut-off	AUC	Sensitivity %	Specificity %	Accuracy %
Dopamine (ng/ml)	0.2	0.85	90	70	83.3
Serotonin (ng/ml)	0.3	0.50	70	80	65.0
BDNF (ng/ml)	85.5	0.92	88	85	87.0

BDNF: brain-derived neurotrophic factor; AUC: Area under the curve.

Abnormalities in the dopamine system especially the mesolimbic dopamine were implicated in social deficits in autism (Chugani, 2011). Although peripheral dopamine level does not necessarily reflect its brain level, it was reported that dopamine agonists improved social performance in studies targeted autistic animals' model. The dopamine low levels could participate in the peripheral symptoms manifested by autistic children such as gastrointestinal disturbances and immune system abnormalities especially T cells malfunctioning (Mannion and Leader, 2016; Careaga *et al.,* 2017). The excess dopamine turnover could explain its low level in blood. Quaak *et al.* (2013) reported elevated homovanillic acid in the urine of autistic individuals which is a substance resulting from dopamine metabolism. The reduced dopamine level could also be related to dopamine transporter functioning which is responsible for the reuptake of dopamine and clearing it from synapses (Cartier *et al.,* 2015). In agreement with these findings, the current study displayed a highly significant decrease in dopamine serum levels among autistic children when compared to normal children.

The correlation detected in the present study between the peripheral levels of dopamine and brain-derived neurotrophic factor could reflect a preserved central relation between them which was previously reported in autistic subjects in the study of Narita *et al.* (2003).

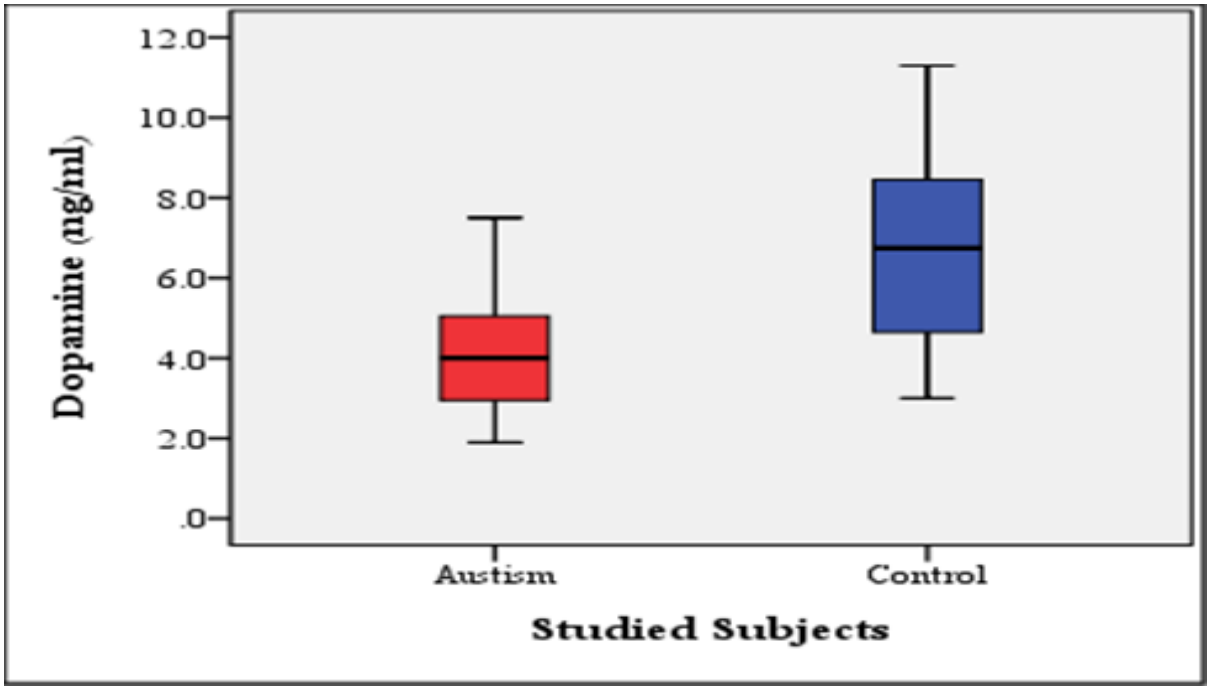

Fig. 1. The levels of dopamine among studied groups.

The brain-derived neurotrophic factor is widely expressed in the hippocampus and hypothalamus. Additionally, receptive language development was linked to the hippocampus these areas are known to be involved in motivation, emotional response, and hormonal balance which were reported to be altered or defective in autism (Miyazaki *et al.,* 2004). This neurotrophin promotes neuronal growth and its optimal level is mandatory for proper connections required for memory, cognition and language development. However, its significantly high levels in the autism group seem to be harmful. Therefore, abnormal BDNF levels could harm brain functioning especially these areas in which BDNF is highly expressed and could have resulted in the negative impact on language abilities.

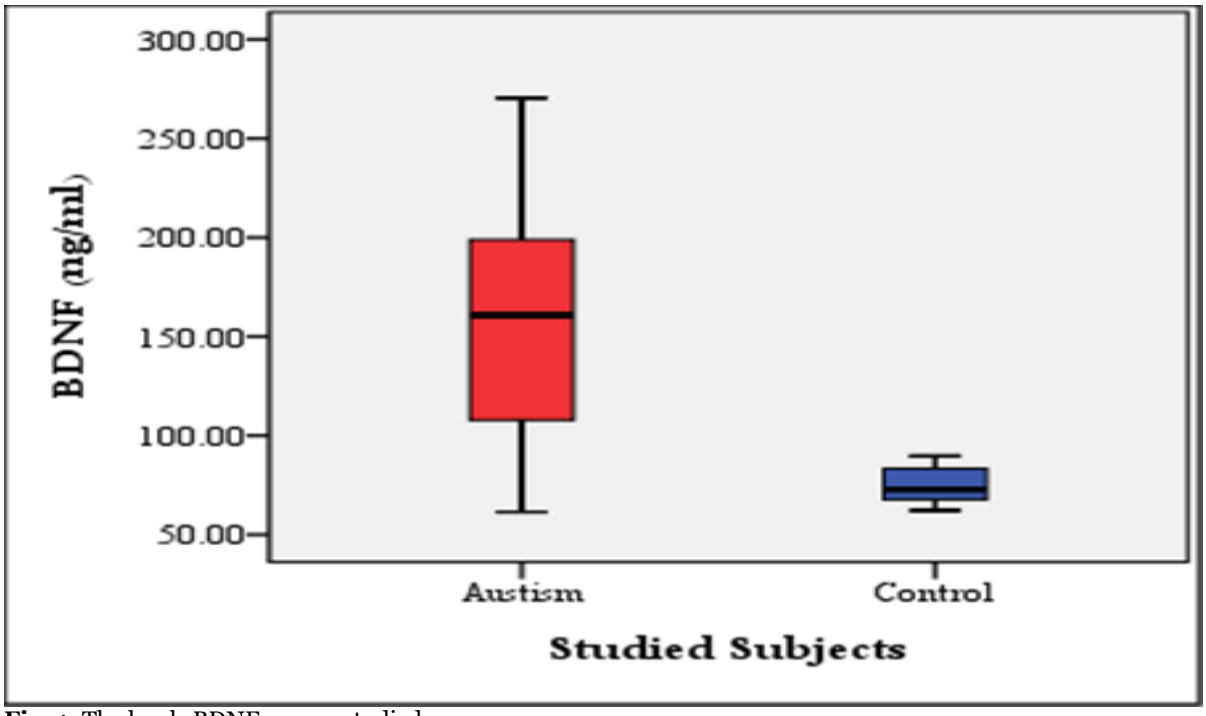

Fig. 1. The levels BDNF among studied groups.

The possible harmful effect of high BDNF levels could be attributed to the imbalance between mature BDNF and the pro BDNF. The pro BDNF role is to inhibit neuronal growth and to induce apoptosis while mature BDNF is responsible for neuronal migration and maturation (Borodinova and Salozhin, 2017).

The cortical thickness in autistic children was reported to be increased and was significantly more than that in typically developing children (Saleh *et al.,* 2015). The high levels of BDNF could have led to excess growth of neurons leading to disturbances in their functioning. The BDNF could have been found to share in the disturbances of the immune system (Lainhart and Lange, 2011). This immune response disturbance adds to the problem related to dopamine deficits and could participate in increasing the severity of autism. Memory disturbances in autism could lead to language delay and hinder verbal and non-verbal communication in autistic children (Toichi and Kamio, 2003).

Episodic memory, for example, helps the child to encode the relations among different stimuli that are related to an event to make this event a cohesive unit. Therefore, this type of memory is very important for social and language development. These abilities were noticed to be defective in autism (Cooper *et al.,* 2017).

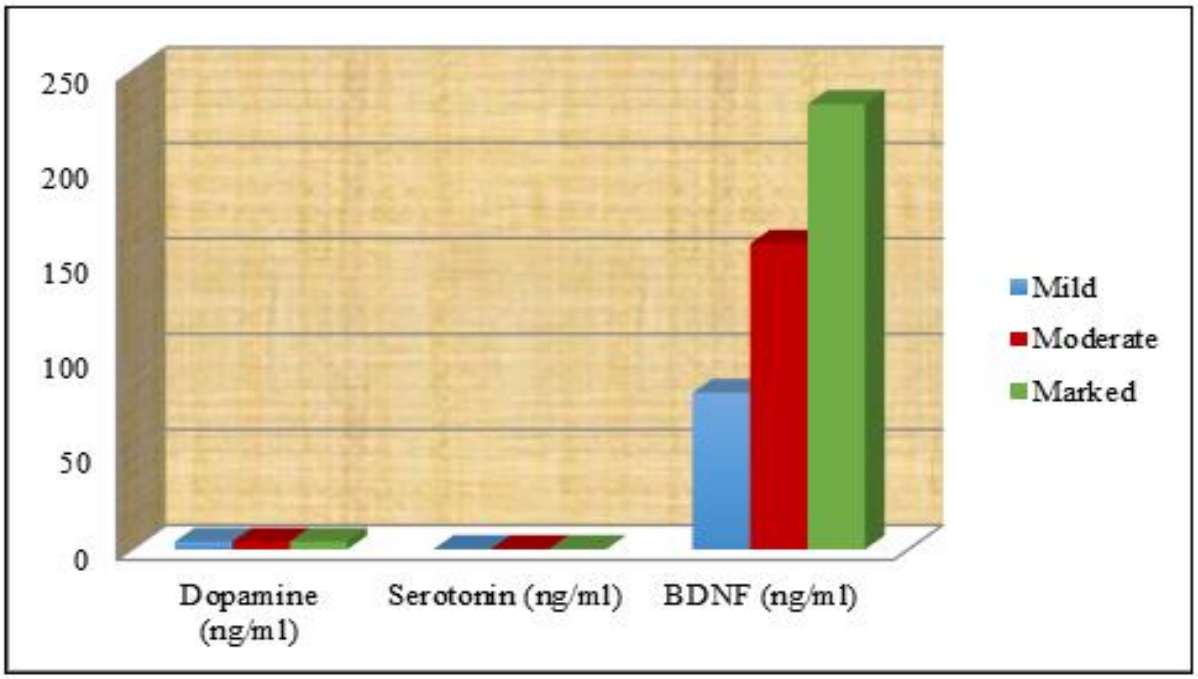

Fig. 2. Frequency of serum dopamine, serotonin and BDNF in different language impairment levels among autistic children.

Deficits in semantics which indicates the meaning of words and sentences were attributed to abnormalities in semantic-episodic memory connection (Toichi and Kamio, 2003). The episodic memory is important for the retrieval of experienced events in the past and it was reported to be defective in autism (Cooper *et al.,* 2017). The BDNF expression has been linked to episodic memory development (Cathomas *et al.,* 2010). Considering all these factors together with the results of the present study which detected a diagnostic accuracy of 83.3% and 87% for dopamine and brain-derived neurotrophic factor respectively, it is advisable to use these measures as biomarkers for such mysterious disorder. Using these biomarkers for the children suspected to have autism could help in the early diagnosis of such children. Furthermore, these measures could be used as follow up markers to monitor the progress in therapy and the response to medication prescribed to them to regain the balance of the biochemical measures in such individuals. Alteration in serotonin levels was previously reported to differ in only 25-50% of cases with autism

(Anderson *et al.,* 2009). However, the absence of significance regarding the serotonin levels in autistic children limits its role in influencing the abilities of this sample of autistic children. These results agree with Ramoz *et al.* (2006) who limits the involvement of the serotonin system in autism.

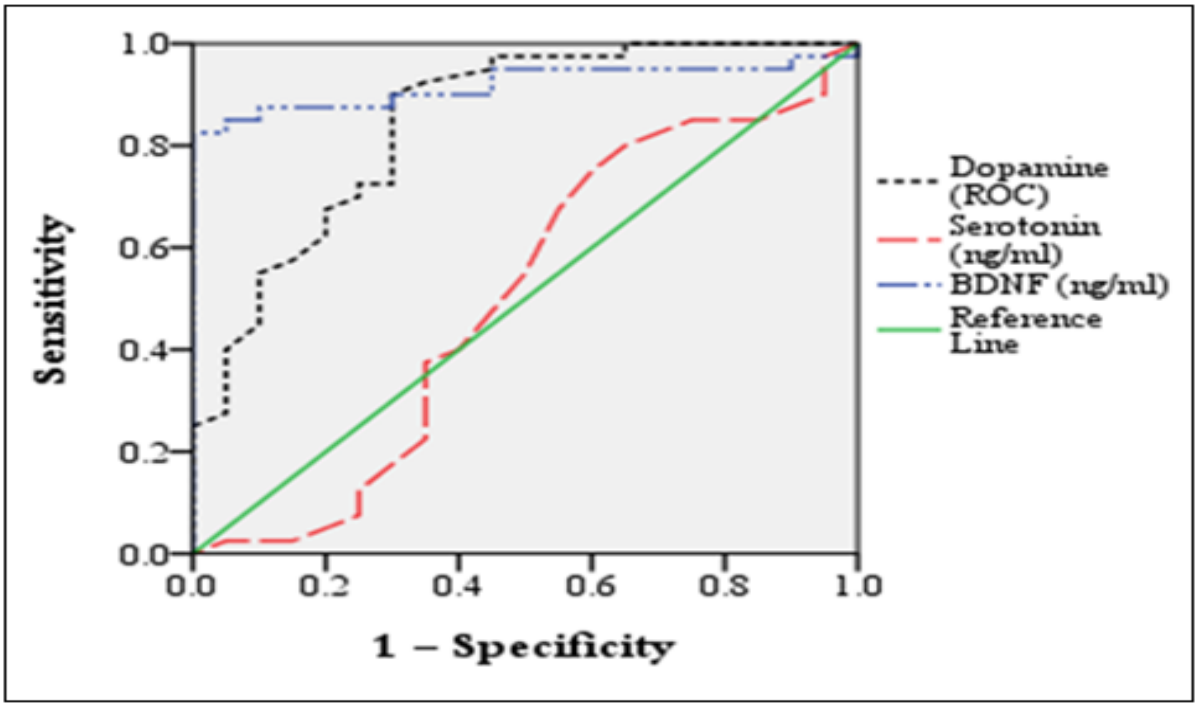

Fig. 3. Receiving operating characteristics (ROC) curve for the dopamine, serotonin and BDNF.

Conclusion

In conclusion, the dopamine and the brain-derived neurotrophic factor could have a role in the pathogenesis of autism. Further, BDNF levels could harm language abilities and thus play a role in the degree of language defect.

These measures could be used as diagnostic biomarkers for children suspected to have autism and for monitoring language impairment.

Declarations

Funding

This research received no specific grant from any funding agency in the public or commercial.

Conflicts of interest

All authors declared no potential conflicts of interest.

Ethics approval

All procedures performed in studies involving human participants were following 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.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable.

Availability of data and material

Data are available with authors upon request.

Code availability

Not applicable.

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