

Brain Derived Neurotrophic Factor in Autism

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ABSTRACT

Introduction:	Autism is a pathophysiological process arising from the interaction of an early environmental insult with a genetic predisposition for the disease. Brain-derived neurotrophic factor (BDNF) is a small protein found throughout the central nervous system (CNS) and peripheral blood. Since autism is a neurodevelopmental disorder that begins in childhood and BDNF is important in neurodevelopment, BDNF is of suggestive potential usefulness as sub-diagnostic biological marker of autism.
Aim of the Study:	This study was designed to assess brain serum levels of BDNF in autistic children.
Subjects and Methods:	This is a case control study conducted on 20 drug naive autistic children and 20 normal children as a control group matched with patients group as regard age and sex. The patients were diagnosed according to DSM-IV criteria and were subjected to full history taking, psychiatric, neurological and general examination including head circumference (H.C.). They also subjected to Children Autistic Rating Scale (CARS) to assess severity of autism and Stanford Binet test to assess I.Q. Blood samples were collected to assess BDNF levels.
Results:	The results revealed that there was no statistically significant difference in BDNF between patients and their matched controls. On the other hand, there was statistically significant increase in H.C. measure in patients compared to controls. There were significant positive correlation between BDNF levels and H.C. and significant negative correlation between BDNF levels and age in patients, in contrast to the significant positive correlation between BDNF levels and age in controls group. When each group (patients and controls) was divided into 2 subgroups according to age (below and above 6 years), the difference between the 2 subgroups in each group and between each subgroup and its comparable one in the other group was significant regarding H.C. and BDNF levels.
Conclusion:	There was higher BDNF concentration in patients below age of 6 years than patients above 6 years old and the converse was in the normal control group.

Key words: Brain-derived neurotrophic factor, autism, neurotrophins, head circumference.

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INTRODUCTION

Autism is a pathophysiological process arising from the interaction of an early environmental insult with a genetic predisposition for the disease¹.

Brain-derived neurotrophic factor (BDNF) is a small protein found throughout the central nervous system (CNS) and peripheral blood. BDNF is involved in the survival and differentiation of dopaminergic neurons in the developing brain and plays an important role in the formation and plasticity of synaptic connections². Also BDNF is trophic for serotonergic neurons and abnormalities in serotonin levels are the most common biochemical findings in autism³.

BDNF and NT-3 (neurotrophin-3) are highly expressed in cortical and hippocampal structures and have been linked to the survival and functioning of multiple neuronal populations⁴.

Neurotrophins are unique in exerting their cellular effects through the actions of two different receptors, the Trk receptor (tyrosine kinase) and p75NTR (p75 neurotrophin receptor). BDNF binds to TrkB⁵.

Animal studies suggest that concentrations of BDNF in the CNS and serum are closely correlated; offering the possibility that BDNF concentration in peripheral blood may be useful as a possible biologic marker for autism⁶.

Since autism is a neurodevelopmental disorder that begins in childhood and BDNF is important in neurodevelopment, BDNF is of suggestive potential usefulness as sub-diagnostic biological marker of autism and that the investigation of this role may lead autism investigators in a new direction of research and the development of effective treatment modalities⁷.

There have been great controversies in studying relationship between BDNF and autism. Some studies reported that serum BDNF are significantly reduced in autism than in normal controls^{8,9}. While other studies reported higher serum BDNF levels obtained from autistic children compared with controls¹⁰.

This study was designed to assess levels of BDNF in a sample of autistic children in comparison to a sample of developmentally normal children matched regarding age and sex.

SUBJECTS AND METHODS

It is a cross sectional and a case control study.

1. Subjects.

Patients group:

Consists of 20 patients, who fulfilled the DSM-IV criteria for autistic disorder. The patients were recruited from the Child Psychiatric Clinic of the Institute of Psychiatry of Ain Shams University,

Inclusion criteria:

1. Age ranges from 3 to 12 years.
2. Both sexes were included.
3. Patients were drug naive.

The exclusion criteria include:

1. Patients with history of neurological disorders, history of head injury with loss of consciousness, or any other medical disorders that may affect brain function.
2. Patients who's parents refused to sign a written consent.

Control group:

Consists of 20 non autistic, developmentally normal children with average I.Q. matched with patient group for age and sex.

2. Procedures.

Informed written consent was obtained from parents of patients and control.

In order to confirm the diagnosis, patients were assessed using:

1. Semi structured interview of Institute of Psychiatry, Ain Shams University.
2. General examination including head circumference.
3. Neurological examination.
4. Blood sample to assess BDNF.
5. Children Autistic Rating Scale (CARS) to assess severity of autistic features¹¹. Its Arabic version was used¹².

CARS is a diagnostic assessment method that rates children on a scale from 1 to 4 for various criteria, ranging from normal to severe and yields a composite score of non-autistic mildly autistic, moderately autistic, or severely autistic. The scale is used to observe and subjectively rate fifteen items.

Total CARS scores range from a 15 to 60, with a minimum score of thirty serving as the cutoff for a diagnosis of autism.

6. Stanford Binet IQ Test to assess IQ¹³.

The Stanford Binet Intelligence Test is a standardized test that assesses IQ and cognitive abilities in children and adults aged 2 to 23. Moreover, the Stanford Binet IQ Test is designed to test intelligence in four areas including verbal reasoning, quantitative reasoning, abstract and visual reasoning and short-term memory skills. Arabic version was used¹⁴.

All controls were subjected to the following procedures:

1. General examination including head circumference.
2. Stanford Binet to confirm that they were within average I.Q.
3. Blood sample to assess BDNF.

Collection of the blood sample:

2 ml of blood was taken from each subject between noon and 18:00 pm. Serum sample was allowed to clot for 30 min before spinning in a centrifuge for 15 min at approximately 1000×g and stored at -20 correct until assay in Medical Research Centre of Ain Shams University. Quantitative determination of serum BDNF concentrations were detected using human BDNF immunoassay kits. The human BDNF immunoassay kits were manufactured in Germany–Catalog No. DBDOO, (R and D systems), expired date at 02 Sep. 2009. In Medical Research Center of Ain Shams University, kits were stored at 2-8 correct.

Serum dilution (1/25-1/100) with sample buffer supplied by the kit is required in order to obtain adequate concentration to measure. Equipments used: ELIZA Reader SLT. SPECTRA and microtitration plate washer.

Statistical analysis:

The data were collected and processed to a personal computer. Data analysis was done using SPSS program version 11.0.1. Continuous data were expressed as mean and standard deviation. The quantitative data were compared using student t-test for independent variable and Parson correlation Coefficient test ("r" test) was used to correlate between two independent quantitative data.

P value: Used to indicate the level of statistical significance
P value<0.05: Significant, P<0.001: Highly significant
P>0.05 non significant.

RESULTS

The patients and control groups were matched regarding age and sex. In patients group the age ranged from 3.9 to 9.7 years with the mean of 6.93 and in the control group, the age ranged from 3.8 to 9.5 years with the mean of 6.87, with no statistically significant difference. Each group consisted of 15(75%) male and 5(25%) females. In the patients group there were 12 patients (60%) with comorbid mental retardation, of them there were 8 and 4 patints with mild and moderate mental retardation ,respectively.

Within the patient group, the severity of autistic features were assessed by CARS and the results revealed 8 mild, 5 moderate and 7 severe cases. Our results revealed that total CARS mean was 38. The highest CARS value was 46 in a female patient aged 9.2 years who had comorbid moderate mental retardation and the lowest value was 31 in a male patient aged 5.8 years.

Our results showed that there was no statistically significant difference between patients and control groups regarding BDNF concentration values ($P>0.05$), the results revealed milder increase in control (1434.45 pg/ml) than in patients (1252.5 pg/ml) regarding BDNF concentration values. There was statistically significant increase in head circumference in patients group compared to control group ($P<0.05$) (Table 1).

Table 1: Comparison between patients and control groups regarding head circumference and BDNF.

variables	GROUP	N	Mean	Std. Deviation	P value	Sig.
Head Circumference (percentiles)	Patient	20	53.100	0.9979	<0.05	S
	Control	20	52.045	1.005		
BDNF concentrations pg/ml	Patient	20	1252.5000	773.126	>0.05	NS
	Control	20	1434.4500	635.251		

S: Significant NS : Non significant

Also the study showed that the highest BDNF concentration was 2250-pg/ml at age 4.1 years old and the lowest BDNF was 340-pg/ml at age 8.5 years old within the patients' group, while in control group, the highest BDNF concentration was 2300-pg/ml at age 6.5 years old and the lowest BDNF was 689 pg/ml at age 3.8 years old.

Each group was then divided into two subgroups, according to age; below and above 6 years. There were highly significant difference between the 2 subgroups of patients group and also the 2 subgroups of control group regarding H.C. and BDNF concentrations (Tables 2 and 3)

Table 2: Comparison between subgroups of patients regarding HC and BDNF.

	Patients below 6 years no=7		Patients above 6 years no=13		P value	Significance
	Mean	SD	Mean	SD		
HC	52.028	0.555	53.676	0.626	<0.001	H. S.
BDNF	2041.428	291.857	827.692	587.56	<0.001	H. S.

H.S. : Highly significant.

Table 3: Comparison between subgroups of controls regarding HC and BDNF.

	Controls below 6 years no=8		Controls above 6 years no=12		P value	Significance
	Mean	SD	Mean	SD		
HC	51.037	0.789	52.716	0.345	<0.001	H. S.
BDNF	877.875	144.367	1804.750	556.880	<0.001	H. S.

H.S. : Highly significant.

There was no significant difference regarding IQ between the two subgroups ($p>0.05$). Also there was no significant difference regarding the mean CARS between them ($P>0.05$).

Comparison between subgroups of patients and controls below age of 6 years showed that there was statistically significant difference regarding the H.C. ($P<0.05$) and also highly statistically significant difference regarding BDNF concentration ($P<0.001$). In addition, comparison between subgroups of patients and controls above age of 6 years showed highly statistically significant difference regarding H.C. and BDNF ($P<0.001$) (Table 4).

Table 4 : Comparison between patients and controls subgroups regarding the head circumference (HC) and the BDNF.

		Patients		Controls		P value	Significance
		Mean	SD	Mean	SD		
Below 6 years	HC	52.023	0.555	51.037	0.789	<0.05	S.
	BDNF	2041.42	291.857	877.875	144.36	<0.001	H.S
Above 6 years	HC	53.676	0.626	52.716	0.345	<0.001	H.S
	BDNF	827.69	587.56	1804.75	556.88	<0.001	H.S

S.: Significant. - HS: Highly significant

Our results showed that in patients group, there were significant positive correlation between BDNF and HC and significant negative correlation between BDNF and age ($P<0.05$) (Table 5).

There were no significant correlation between BDNF and CARS (severity of autistic features) ($P>0.05$) and no significant correlation between BDNF and IQ (Table 5).

Table 5: Correlation between BDNF and other parameters in patients group.

	Pearson correlation (r)	p value	Significance
Age	-0.795	<0.001	HS
IQ	-0.062	>0.05	NS
CARS	-0.227	>0.05	NS
H.C	+0.621	<0.05	S

S: Significant - NS: Non significant - HS: Highly significant

Also, there was no significant correlation between the score of CARS and age nor H.C. (Table 6).

Table 6: Correlation between the scores of CARS and other parameters in patients group.

	Pearson correlation (r)	P value	Significant
Age	+0.42	>0.05	NS
Head circumference (HC)	+0.32	>0.05	NS

NS: Non significant

In control group, there was a significant positive correlation between BDNF and age and no significant correlation between BDNF and H.C. ($P>0.05$) (Table 7).

Table 7: Correlation between BDNF and other parameters in control group.

	Pearson correlation (r)	P value	significance
Age	+0.523	0.011	HS
H.C	+0.073	0.782	NS

DISCUSSION

BDNF has a possible role in the pathogenesis of autism through its neurotrophic effects on the serotonergic system. The pathophysiological correlation between BDNF and autism are not well explained yet^{15,16}.

Our results revealed that among the autistic group, 75% were males and 25% were females. These results are in agreement with numerous studies showing that gender distribution in autistic disorder is three to four times more frequent in boys than in girls¹⁷. There were 12 patients (60%) having comorbid mental retardation (MR), of them 8 and 4 with mild and moderate mental retardation (MR), respectively. These results are in line with studies reported that approximately 70% of children with autistic disorder have MR¹⁸.

Regarding BDNF concentration values, there have been great controversies in studying relationship between BDNF and autism. In the current study, there was no significant difference between serum level of BDNF in the autistic group compared to the age and sex matched children of the control group. This finding is in line with the study of Connolly et al. Which found that no significant difference regarding serum levels of BDNF was found between children with autism and children with normal development (18 patients and 18 controls)¹⁹.

Some studies showed a higher level of BDNF among autistics patients compared to controls²⁰. The disagreement can be explained by methodological difference as the elevated levels were found in postmortem brain tissue not serum BDNF levels.

Also, our results disagreed with the study of Lujan which showed that expression of “BDNF in the drug-naïve autistic group was significantly higher than in the control group. The possible explanation for contradicting results is that his patients and controls were not matched for age²¹.

Serum concentration of BDNF and neurotrophin-4(NT-4) had been measured in patients diagnosed with autism and compared to the control group and found to be higher in autistic group. The study was composed of 18 autistic patients with mean age 7.6 years (17 males and 1 female) and 16 controls with mean age 23.3 years (5 males and 11 females)²¹. The disagreement may be explained due to difference in the mean age (non matching between patients and control).

Other studies examined the expression of BDNF mRNA in the peripheral blood lymphocytes of drug-naïve autistic

patients and control subjects showing significantly higher. BDNF expression in the drug-naïve autistic group compared to the control group¹⁰.

On the contrary other studies have reported significantly reduced BDNF in autistics than in normal controls⁸. The disagreement may be explained by the presence of epilepsy as a comorbidity in 6 autistic patients.

Also Hashimoto et al.²² reported that serum BDNF are significantly reduced in autism than in normal controls²². This disagreement may be explained by that the patients were not drug naïve.

The regulation of BDNF in the cerebellum of six autistic patients and six controls was studied by measuring the protein level of BDNF in post mortem tissues. The level of BDNF was significantly decreased in the autistic group compared to controls. Reduced BDNF in the cerebellum may be an indicator of aberrant brain development and growth in autism²¹.

The controversies between mentioned studies might point out BDNF as an important factor rather than being the only potential biological marker for autism.

BDNF is one of major neurotrophins responsible for brain growth and development during pregnancy and early infancy²². During the first years of life, head circumference does correlate well with brain size in typically developing and autistic children²³ and it has been used as a retrospective indicator of relative brain size in autism. Farley stated that brains of autistic children undergo an abnormal and dramatic growth spurt in the first year of their lives. The growth makes their heads markedly larger than those of normal infants^{25,27}.

In the current study, there was significant increase in head circumference in patient group compared to control group these results generally agree with Courchesne et al. Who compared the growth patterns of autistic children's heads with those of normal children. Finding that by 6-14 months of age, autistic children's head circumferences were substantially larger than normal children²⁴.

Due to the fact of controversial results of BDNF in autistics in previous studies as well as due to observed wide variation in BDNF levels in both patients and control groups, we divided each group into 2 subgroups according to age. The comparison between both subgroups revealed that patients below age of 6 years had significantly higher levels of BDNF than patients above age of 6 years. This was confirmed by another the significant negative correlation between BDNF and age ($P < 0.05$). These results are in line with the study of Gilmore which revealed more increase in BDNF levels in earlier ages of autistic children than in older ages²⁷.

In addition these results are supported by the finding that serum BDNF levels are increased in autistic children below 6 years compared with those above 6 years²⁶.

This was contrary to normal control group where those subjects below 6 years old had significantly lower BDNF levels than those above 6 years. Also there was significant positive correlation between age and BDNF levels in control group.

There was significant difference between patients and control groups regarding head circumference ($P < 0.05$) and a positive correlation between BDNF concentrations and head circumference in patients with autism. Our results agree with the study of Gillberg and De Souza which revealed significant increase of head circumference in 50 autistic children compared to 50 non-autistic children²⁸.

There was no significant correlation between BDNF and CARS (severity of autistic features) ($P > 0.05$) and no significant correlation between BDNF and IQ. These results are in agreement with other studies that showed that no correlation between BDNF and severity of autistic features (assessed by CARS); also, no correlation between BDNF concentrations and IQ within the patient groups²². That is to say that BDNF concentration is not correlated neither to severity of autism nor to intelligence.

Under the light of these results we conclude that age is negatively correlated to BDNF levels in autistic patients (the reverse in normal children) and this reflected as increase in the H.C. Which is positively correlated to BDNF levels. This leads us to deduce that there is an abnormal status of prenatal and an early post-natal development in autistic children and that BDNF is an important marker but not the only one in this process. This also indicates that H.C. is an important measurement since birth.

This study needs to be replicated using large sample. Also follow up study is recommended to detect variations in BDNF levels with changes in age. And to compare BDNF levels in drug naïve autistic patients to medicated patients to assess effect of drugs on BDNF levels and possible role of BDNF as predictor of response.

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الملخص العربي

العامل المحفز للنمو العصبي المشتق من المخ في اضطراب الذاتوية

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تم تصميم هذه الدراسة لتقييم دور العامل المحفز للنمو العصبي المشتق من المخ في مرضى اضطراب الذاتوية . وقد تمت هذه الدراسة على ٢٠ مريض مصاب بمرض اضطراب الذاتوية من مرضى العيادة الخارجية لطب نفس الأطفال بمركز الطب النفسى بجامعة عين شمس وشملت العينة الضابطة ٢٠ طفل طبيعى مماثلين من حيث العمر والنوع. وقد تم إستخدام الأدوات التالية: تشخيص المرضى حسب التقييم الأمريكى للإضطرابات النفسية الرابع , فحص إكلينيكي و يشمل محيط الرأس, مقياس كارز, مقياس إستانفورد بينيه وعينة من الدم لقياس نسبة العامل المحفز للنمو العصبي المشتق من المخ.

لقد أسفرت النتائج بأنه لم يوجد فارق احصائي فى نسب العامل المحفز للنمو العصبي المشتق من المخ بين مجموعة المرضى والمجموعة الضابطة، ولكن وجد فارق احصائي بالنسبة لمحيط الرأس فى مجموعة المرضى عنه فى المجموعة الضابطة. ولكن عند تقسيم كل مجموعة الى مجموعتين حسب السن: مجموعة تحت ٦ سنوات ومجموعة فوق ٦ سنوات وجد أنه يوجد فارق إحصائي فى نسبة العامل المحفز ومحيط الرأس بين مجموعات المرضى والمجموعات الضابطة. وقد أوضحت النتائج بوجود علاقة عكسية بين أعمار المرضى ونسب العامل المحفز للنمو العصبي، ووجود علاقة طردية بين محيط الرأس لدى الأطفال المرضى ونسب العامل المذكور.