

Plasma brain-derived neurotrophic factor (BDNF) in children with attention deficit-hyperactivity disorder (ADHD).

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Summary

Background: ADHD is a common childhood disorder with serious effect on the patient and his Family's life. Recent evidence suggests a relation between ADHD and BDNF a neurotrophin responsible for neuronal plasticity and act as a neurotransmitter modulator.

Aim of the study: To know the possible relation between BDNF level and ADHD so it could be used as a potential marker for this disorder.

Subjects and methods: Fifty three cases of ADHD were selected 29 patients were drug naive and 24 patients were Receiving pharmacological treatment. The ADHD cases underwent IQ test (WISC), Conner's test to assess severity of different symptoms, 3 ml blood sample before noon were collected to measure plasma level of BDNF. After full assessment patients were categorized into 3 diagnostic categories (hyperactive impulsive type, inattentive type, and combined type). Control group consisted of thirty normal volunteer children, with no psychiatric or neurological disorder. 3ml non clotted blood samples were collected from them before noon.

Results: In our study the mean plasma BDNF levels were significantly higher in ADHD patients than in normal controls (p value was 0.010). Conclusion: Our study suggests that there is an increase of plasma BDNF levels in ADHD patients.

مستوى العامل التغذوي العصبي بلازما الدم في الأطفال المصابون بفرط الحركة ونقص الانتباه

الخلفية: اضطراب فرط الحركة ونقص الانتباه هو مرض ذو تأثير خطير على المرضى وعائلاتهم. تشير الأدلة الحديثة إلى العلاقة بين الاضطراب والعامل التغذوي العصبي وهو احد المغذيات العصبية المسئولة عن مرونة العصبية كما انه يعمل كمغير للموصلات العصبية.

الهدف من الدراسة: معرفة العلاقة المحتملة بين العامل التغذوي العصبي واضطراب فرط الحركة حتى يتمكن من معرفة امكانيه استخدامه كمحدد للمرض.

خطه البحث: تم اختيار 53 حالة مصابه بفرط الحركة ونقص الانتباه 29 منهم لا يتناولون اي علاج دوائي و 24 مريض يتناولون العقاقير الطبيه. تم عمل اختبار وكسلر لقياس ذكاء الأطفال واختبار كونورز لقياس شدة الاعراض المختلفه وتم سحب 3 ملل بلازما من المرضى قبل الظهر. تم تقسيم المرضى بعد التقييم الشامل الى ثلاث مجموعات: مرضى يعانون من فرط الحركة والاندفاع ومرضى يعانون نقص الانتباه ومرضى يعانون من نقص الحركة وفرط الانتباه معا. تتكون المجموعه الضابطه من 30 طفل طبيعى متطوع.

النتائج: تظهر هذه الدراسه ان متوسط العامل التغذوي العصبي في بلازما الدم اعلى في اضطراب فرط الحركة ونقص الانتباه من يتناولون اي ادويه.

Introduction:

Attention-deficit hyperactivity disorder (ADHD) has been identified as an important psychiatric condition in terms of its prevalence (around 5% worldwide) and its impact on quality of life for patients and their families (Cho et al., 2010). Also ADHD is the most commonly diagnosed behavioral disorder of childhood (American Academy of Pediatrics, 2000).

Attention-deficit/hyperactivity disorder (ADHD) is characterized by hyperactivity, diminished sustained attention and higher levels of impulsivity in a child or adolescent than expected for someone of that age and developmental level (Sadock and Sadock, 2007). These core behavioral symptoms must be pervasive across situations, persistent for more than 6 months and observed before the age of 7 years, as defined by the diagnostic and statistical manual of mental disorders (DSM-IV-TR) issued by (American Psychiatric Association, 2000).

These behavioral manifestations contribute to diminish academic, occupational and social functioning, and have neurobiological bases. (De La Fuente A, 2013). 30 to 50% of those individuals diagnosed in childhood continue to have symptoms into adulthood. As they mature (Bálint et al, 2008).

The etiology of ADHD is now viewed to be pathophysiological and clinically heterogeneous entity, hypotheses on the etiology of ADHD have evolved from simple one-cause theories to multi-factorial processes that reflect the confluence of many types of risk factors, including genetic, neurochemical, environmental and psychosocial factors (Biederman and Faraone, 2005).

Genetic research on ADHD started with the finding that hyperactivity tends to aggregate in families since then, family studies have shown that ADHD shows familial clustering both within and across generations. Increased rates of ADHD among the parents and siblings of ADHD children have been observed (Franke et al., 2012).

Evidence from various sources suggests primary involvement of the dopaminergic system. Molecular genetic studies also indicate a linkage of genetic polymorphisms in the dopaminergic system, such as dopamine D4 and D5 receptors, and dopamine transporter (DAT), to ADHD (Bobb et al., 2005).

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that modulates different aspects of neuronal function during development and in the mature nervous system (Gottmann et al., 2009). Recent reports have suggested a pathophysiological role of BDNF in ADHD (Shim et al., 2008).

It acts through tropomyosin-related kinase B (TrkB) receptors either pre- and postsynaptically to modulate long-term potentiation (LTP), which serves as a molecular model for the synaptic events underlying memory formation (Farmer et al., 2004). Furthermore, BDNF enhances glutamatergic synaptic transmission (Gottmann et al., 2009) and has been strongly implicated in spatial learning (Yamada et al., 2002).

First, earlier studies demonstrated that BDNF plays a key role in the survival and differentiation of midbrain dopaminergic neurons *in vivo* (Hyman et al., 1991) and *in vitro* (Spina et al., 1992).

Since dysfunction in the midbrain system is crucial in ADHD pathogenesis (Solanto, 2002), a decreased midbrain BDNF activity may cause midbrain dopaminergic dysfunction, and therefore, resulting in ADHD. Second, psychostimulants such as methylphenidate are the agents commonly used in the treatment of ADHD. The classical action mechanism of psychostimulants

involves enhancement of the release of dopamine and norepinephrine in the midbrain. BDNF has been shown to modulate the release of dopamine through activation of TrkB (tropomyosin-related kinase B) receptors (Blochl and Sirrenberg, 1996) and has also been implicated in the release of dopamine as well as in dopamine-related behaviors induced by psycho-stimulant, methamphetamine (Narita et al., 2003).

Subjects And Methods:

The present study included 83 subjects; thirty healthy subject of both sexes representing the control group, 29 new ADHD cases who have received no medical treatment (drug naive) of both sexes and 24 ADHD cases who have taken pharmacological treatment for ADHD of both sexes.

The patients were recruited from the regular attendants of the psychiatric Clinic in the institute of post graduate childhood studies and Institute of Psychiatry, Ain Shams University hospitals. The Controls were recruited from the child health clinic of the National research center.

Cases were included according to; Age from (6-15) years and Cases being diagnosed with ADHD according to the diagnostic Criteria for Attention-Deficit/Hyperactivity Disorder of the Diagnostic and statistical manual of psychiatric disease; Cases with Full scale IQ lower than 70, Bipolar affective disorders, Neurological and seizure disorders, Pervasive developmental disorders, Chronic tic disorder and Tourette's disorder, Psychotic disorders, were excluded. All patients and controls other than group 2 have not been receiving any medications for at least one month prior to their participation in the study. Written informed consent was obtained from parents after explanation of the aim and procedures of the study.

All studied children were subjected to; Full medical history and clinical examination. Full psychiatric history and mental status examination using the semi-structured interview to the psychiatric sheet of the institute of post graduate childhood studies. Ain shams university, Auxological Assessment: Growth was assessed through auxological measurements of weight, height and head circumference. Assessment of cognitive function: The Arabic version of the Revised Wechsler Intelligence Scale for Children (WISC-R) (Wechsler, 1977) (Kamel & Ismaiel, 1993), The Benton visual retention test; to assess memory (Benton, 1974). The Conners' parent Rating Scales- Revised (CRS-R) long version was completed by parents to assist in evaluating children for attention deficit/hyperactivity disorder (ADHD) (Conner's, 1997). Laboratory investigations; For ADHD patients and normal controls, blood sample was drawn from the subjects under complete aseptic conditions between 8 a. m. and noon to exclude the circadian effect of BDNF levels (Begliuomini et al., 2008). Approximately 5 ml of plasma was collected on ice in polypropylene tubes using citrate as an anticoagulant. Samples were centrifuged for 15 minutes at 1000×g at 2-8°C within 30 minutes of collection, then separated plasma was additionally centrifuged at 10,000×g for 10 minutes at 2-8°C. samples were then stored at -20°C till assay.

The assay employed the quantitative sandwich enzyme immune assay technique. A monoclonal antibody specific for BDNF has been pre-coated onto a micro plate. Standards and samples were pipetted into the wells and any BDNF present was bound by the immobilized antibody. An enzyme linked monoclonal antibody specific for BDNF was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of BDNF bound in the initial step. The intensity of the color was

measured.

Quantitative determination of plasma BDNF concentrations were detected using human BDNF immunoassay kits. The human BDNF immunoassay kits were manufactured in USA- Catalog No. DBD00 SBD00 PDDBD00, (R and D systems), expiry date at 24 October. 2012. In the National research center labs; kits were stored at 2-8° C. plasma dilution (1/ 20) with sample buffer supplied by the kit is required in order to obtain adequate concentration to measure. Equipment used: ELIZA Reader SLT. SPECTRA and micro titration plate washer.

Statistical Analysis:

Statistical analysis was done on a personal computer using IBM© SPSS® Statistics version 21 (IBM© Corp., Armonk, NY).

Categorical data are presented as number and percentage and between-group differences are compared using the Pearson chi square test or the chi square test for trends for nominal or ordinal data, respectively. Fisher's exact test is used in place of the chi square test if > 20% of cells in any contingency table had an expected count of < 5. Normality of numerical data distribution was tested using The D'Agostino- Pearson test. Non- normally distributed numerical data are presented as quartiles, minimum, and maximum. Normally distributed data are presented as mean and SD, and intergroup differences are examined using the unpaired Student t test was (for 2 independent groups) or one- way analysis of variance (ANOVA) (for multiple groups).

Correlations among numerical variables are examined using the Pearson moment- product correlation analysis. The Spearman rank correlation is used for ordinal variables. All P values are two- sided. P <0.05 is considered statistically significant.

Results:

Table (1) Comparison between patients and healthy controls as regard age, Weight (kg), Weight for age (percentile), Height (cm), Height for age (percentile), BMI (kg/m²), BMI for age (percentile), Head circumference (cm).

General Characteristics	Patients (Mean)	Controls (Mean)	Patients (SD)	Controls (SD)	P Value
age	9.0	9.2	2.6	2.3	0.779
Weight (Kg)	31.9	31.8	10.5	11.1	0.959
Weight for age (percentile)	59.5	58.1	26.3	26.7	0.809
Height (Cm)	130.9	132.9	13.8	11.6	0.482
Height for age (percentile)	45.0	49.0	26.0	26.4	0.515
BMI (kg/m ²)	18.0	17.5	2.7	3.0	0.479
BMI for age (percentile)	71.6	63.7	17.8	26.1	0.148
Head Circumference (Cm)	53.4	52.6	2.8	1.9	0.128

Table (1); shows non- significant difference between ADHD patients and controls as regard age, Weight (kg), Weight for age (percentile), Weight for age (Z- score), Height (cm), Height for age (percentile), Height for age (Z- score), BMI (kg/m²), BMI for age (percentile), BMI for age (Z- score), Head circumference (cm). P<0.05 is significant. P>0.05 is non- significant. BMI= body mass index. SD= standard deviation.

Table (2) Comparison between patients and healthy controls as regards; Gender, Consanguinity and Handedness.

General Characteristic	Gender				P- Value	Consanguinity				P- Value	Handedness				P- value
	Male		Female			No Consanguinity		Consanguinity			Right		Left		
	No	%	No	%		No	%	No	%		No	%	No	%	
Cases	41	77.4	12	22.6	0.011	33	62.3	20	37.7	0.094	47	88.7	6	11.3	1.0
Controls	15	50.0	15	50.0		24	80	6	20		27	90	3	10	

Table (2) shows significant difference between cases and controls as regard sex; out of 53 patients 41 were males (77.4%) while in the control group 15

were males (50%). As regard consanguinity and handedness no statistical difference was found between cases and controls. P <0.05 is significant. P>0.05 is non- significant.

Table (3): Comparison between patients and controls as regard perinatal conditions& infancy

		Controls		Cases		P- Value
		N	%	N	%	
Pregnancy	Uneventful	N	24	42	0.082	
		%	80.0%	79.2%		
	GDM	N	1	5		
		%	3.3%	9.4%		
	PE	N	5	2		
		%	16.7%	3.8%		
Bad Emotional Status	N	0	4			
	%	0.0%	7.5%			
Delivery	SVD	N	17	29	0.864	
		%	56.7%	54.7%		
	CS	N	13	24		
		%	43.3%	45.3%		
Postnatal Course	Normal	N	21	33	0.751	
		%	70.0%	62.3%		
	Neonatal Jaundice	N	7	17		
		%	23.3%	32.1%		
	Admission To NICU	N	2	2		
		%	6.7%	3.8%		
	Cyanosis	N	0	1		
		%	0.0%	1.9%		
Infancy	Normal	N	23	39	0.921	
		%	76.7%	73.6%		
	Difficult Child	N	5	11		
		%	16.7%	20.8%		
	Delayed Milestones	N	2	3		
		%	6.7%	5.7%		

Note: GDM= gestational diabetes mellitus, PE= preeclampsia, SVD= spontaneous vaginal delivery, CS= caesarian section, NICU= neonatal intensive care unit.

Table 3; shows non- significant difference between cases and controls as regard pregnancy, delivery, postnatal conditions and infancy. P <0.05 is significant. P>0.05 is non- significant.

Table (4): comparison between cases and Controls in Wechslerintelligence scale and Benton visual retention test

Variable	Controls No= 30		Cases No= 53		P- Value
	Mean	SD	Mean	SD	
WISC: Verbal Subscales	107.3	8.8	95.5	11.2	<0.001
Wis: Comprehension	11.6	2.6	9.8	2.3	0.002
Wis: Arithmetic	10.8	1.8	8.6	2.3	<0.001
Wis: Similarities	10.5	2.2	8.8	2.9	0.004
WISC: Digit Span	8.8	1.7	7.6	2.0	0.008
WISC: Performance Subclasses	102.1	7.1	90.8	12.0	<0.001
WISC: Picture Completion	10.0	1.5	8.4	1.7	<0.001
WISC: Block Design	8.7	1.3	7.8	1.8	0.018
Wis: Coding	10.3	1.9	8.4	2.5	<0.001
Wis: Total Iq	104.8	7.9	92.6	11.5	0.000
BVRT: O- E correct score	1.2	0.9	2.0	1.5	0.003
BVRT: O- E error score	1.9	1.3	3.0	2.3	0.008

Note: WISC= Wechsler intelligence scale for children, IQ= intelligence quotient, BVRT= Benton visual retention test, O- E= difference between observed score and expected score.

Table 5; shows significant statistical difference between ADHD cases and healthy controls as regard Wechsler intelligence subscales and total IQ. Also showing statistically significant difference between cases and controls in Benton visual Retention test scores. P<0.05 is significant. P>0.05 is non- significant.

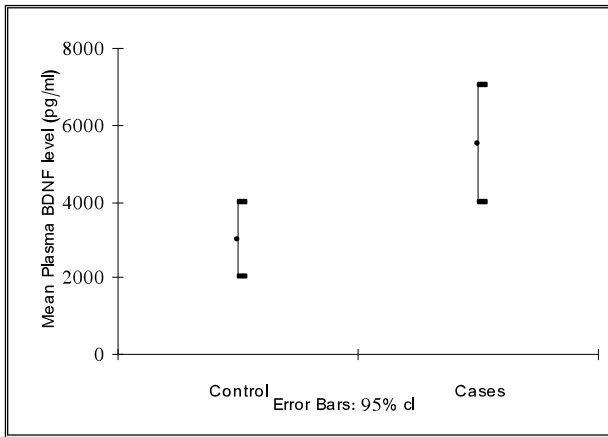


Figure (1) Comparison between Serum BDNF level between Cases and Controls
 Note: BDNF= Brain derived neurotrophic factor, CI= confidence interval.

Figure (1) showing significant increase in serum BDNF level among cases more than controls. Mean plasma BDNF level in controls is 3138.5, and standard deviation is 2640.7. While mean plasma BDNF in cases is 5476.9 and standard deviation is 5443. p value is 0.010 P <0.05 is significant. P>0.05 is non-significant.

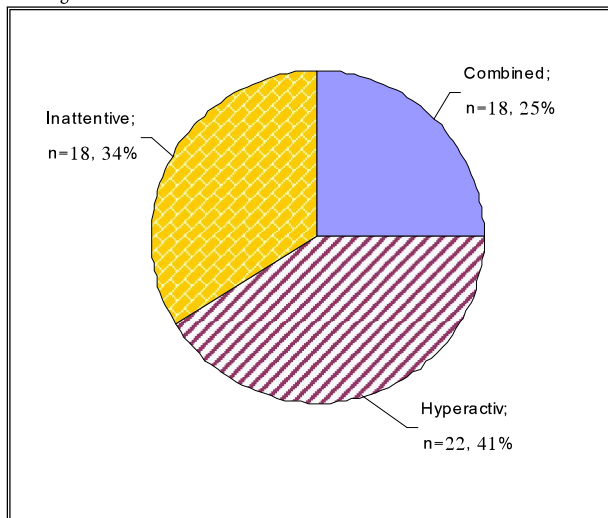


Figure (2): prevalence of ADHD subtypes in study population.
 Note: ADHD= attention deficit hyperactivity disorder. N= number

Figure (2) showing 22 out of 53 cases were hyperactive impulsive type representing 41.5% of the study population, 18 patients were inattentive representing 34%, 13 patients were of the combined type representing 25%.

Discussion:

The aim of the present study was to find the relation between plasma BDNF level and ADHD and to investigate the effect of pharmacological therapy on ADHD. In the present study, we investigated the levels of plasma BDNF in children with ADHD and found that plasma BDNF levels were significantly higher in children with ADHD than in normal controls. Our results came in accordance with the findings of Shim and his colleagues (the first study to investigate the relation between BDNF and ADHD in children); they found that the mean plasma BDNF levels in ADHD patients were 833.8±371.0 pg/ml, whereas 578.5±304.0 pg/ml in normal controls, thus showing significantly higher mean plasma BDNF levels in ADHD patients than in normal controls (Shim et al., 2008).

However the results of Sayyah disagree with our results and found no significant difference between plasma BDNF level in both cases and controls

(For control children BDNF plasma level was mean= 28689pg/ml, SD+/- 12705 and the mean BDNF level 27171 pg/ml, SD+/- 25368.5 (Sayyah, 2009).

regarding anthropometric measures weight for age height for age and head circumference there were no significant statistical difference between cases and controls this contradicts with (Holtkamp, 2004; Pagoto et al., 2009; Waring and Lapane, 2008) Who found significant relation between obesity and ADHD patients than controls this may be due to the large sample size used in these studies. Bahrami stated that children with ADHD face a significant risk of becoming overweight and that there are also significant risks that overweight children may have ADHD (Bahrami, 2013). Also Yang and colleagues studied A total of 158 Chinese child with ADHD. The prevalence of obesity, overweight, were 12.0%, 17.1%, respectively, which were significantly higher than in the general Chinese population (2.1%, 4.5%, respectively). Multivariable analysis showed that the children with the combined subtype of ADHD and the onset of puberty were at a higher risk of becoming obese or overweight (Yang et al., 2013) another reason why our study may contradict with other studies is the racial difference Yang found the prevalence of overweight and obesity in control children to be 4.5 and 2.1% respectively In Egypt the prevalence of overweight and obesity is 12.1 and 6.2% respectively according to (Salazar- Martinez et al., 2006).

In our study out of 53 patients 41 were males (77.4%), 12 were females representing (22.6%) while in the control group 15 were males (50%) and 15 were females (50%). This comes in accordance with Jamison who stated that ADHD is much more common among males than females; boys are two to three times more likely to have ADHD than girls. They are up to nine times more likely than girls to be referred for evaluation and treatment. The difference in referral rates between ADHD boys and girls is likely due to ADHD boys having more behavior problems than ADHD girls (Jamison, 2000).

As for consanguinity our results showed non-significant statistical difference between cases and controls regarding parental consanguinity this contradicts with Al-Sharbaty and colleagues who found that The contribution of consanguinity and a history of acquired brain injury to be common features (Al-Sharbaty et al., 2011).

Handedness was studied and we found that there were no significant difference between patients and controls regarding handedness, this was consistent with Ahmad Ghanizadeh who stated that Left- or right- hand preference was not associated with age, gender, inattentiveness score, hyperactivity- impulsivity score, comorbid psychiatric problems, developmental coordination problems score, or parental characteristics (Ghanizadeh, 2013).

In our study we also studied the effect of perinatal factors in the etiology of ADHD and we found non-significant statistical difference between cases and controls with this regards. This contradicts with Ketzer et al, who found that using Conditional logistic regression analysis children and adolescents whose mothers presented more Prenatal, delivery and early post- natal problems had a significantly higher risk for ADHD (Ketzer et al., 2012), the study of Ketzer and colleagues Perinatal complications were assessed by direct interview with biological mothers and supplemented with medical records when possible. Amor et al. found greater number of neonatal complications in their sample of 50 ADHD children compared with their 50 unaffected siblings. No differences between cases and controls related to low birth and to maternal alcohol and

tobacco consumption during pregnancy were found (Amor et al, 2005). In another study, disruptive behavior disorders were significantly associated with maternal physical problems during pregnancy and delivery, especially acute anoxia/hypoxia (Allen et al.,1998). The difference between our results and these previous studies may be because they applied several different scales and also due to the fact that maternal smoking, a very important prenatal risk factor was not found in our study.

We studied the effect of ADHD on IQ; total scores and subscales, by comparing cases and controls ADHD cases had lower scores in all scores total, performance subscale and verbal subscale; the mean of the Verbal subscales in controls was 107.3+/- 8.8 while in Cases it was 95.5+/- 11.2. The performance subscales; mean value in controls is 102.+/- 17.1 while cases mean value was 90.8+/- 12.0. This comes in accordance with Fraiser and colleagues who stated that Effect sizes for overall intellectual ability (Full Scale IQ) were significantly different between ADHD and healthy participants. Effect sizes for Full Scale IQ were significantly smaller than those for spelling and arithmetic achievement tests and marginally significantly smaller than those for continuous performance tests but were comparable to effect sizes for all other measures. These findings indicate that overall cognitive ability is significantly lower among persons with ADHD and that Full Scale IQ may show as large a difference between ADHD and control participants as most other measures (Frazier et al., 2004). Voigt also stated that similar to the general population, children with ADHD may have a broad range of cognitive abilities and reported that ADHD is more likely to be present in the context of developmental delay, at the level of borderline- to- mild intellectual disability (Voigt et al., 2006). On the other hand Kaplan applied full scale Wechsler intelligence scale on 63 children with ADHD, 69 children with reading difficulties (RD), and 68 children with comorbid ADHD+ RD, the distributions of estimated Full Scale IQs for each of the three groups of children did not differ significantly from a normal distribution, with the majority of children (more than 50%) in each group scoring in the average range. The percentage of children with ADHD who scored in the above-average range was not significantly higher than the percentages of children in the other two groups. It was concluded that children with ADHD are no more likely to have an above- average IQ than are other children (Kaplan et al., 2000)

In the present study we found that children with ADHD had significant memory impairment compared with healthy controls, we applied the Benton visual retention test to assess visual perception and visual memory measures visuo- spatial abilities and immediate spatial memory abilities. and we found mean difference between observed and expected true score in controls 1.2+/- 0.9 while in controls the difference was 2.0 +/- 1.5, this comes in accordance with Marzocchi et al. who compared ADHD patients together with normal controls and reading disabled children and found significant immediate memory impairment in ADHD children compared to controls (Marzocchi et al., 2008).

The prevalence of ADHD subtypes in the present study population was 22 out of 53 cases were hyperactive impulsive type representing 41.5% of the study population, 18 patients were inattentive representing 34%, 13 patients were of the combined type representing 24.5%. A meta- analysis by Willcutt stated that reviewing several studies that discussed the prevalence of ADHD subtype When subtypes were defined based only on symptom criteria, ADHD

(inattentive type) was the most common subtype based on parent ratings alone, teacher ratings alone, self- report ratings, and parent- teacher agreement (38-57% of all individuals with ADHD). The relative frequencies of ADHD (combined subtype) and ADHD (Hyperactive subtype) varied by reporter; more individuals with ADHD met the criteria for ADHD (Hyperactive subtype) than ADHD (Combined subtype) based on parent ratings (37% vs. 25%) and self- report ratings (36% vs. 22%), whereas a higher proportion met the criteria for ADHD (Combined subtype) than ADHD (hyperactive subtype) when ADHD was defined by teacher ratings or was in agreement between parents and teachers (24- 30% vs. 19- 20%) (Willcutt, 2012) When applying the full DSMIV criteria; ADHD (inattentive subtype) remained the most common subtype when parent, teacher, or self- report ratings were used to define ADHD based on full DSM- IV criteria, but the rate of ADHD (Combined subtype) was higher than the rate of ADHD (Inattentive) for best estimate diagnoses. The estimated prevalence of ADHD (Hyperactive) was lower than ADHD (Combined) or ADHD (Inattentive) for all algorithms that applied to the full diagnostic criteria (i.e., 13- 17% of all individuals with ADHD), reflecting the lower rates of functional impairment in groups that met symptom criteria for ADHD (Hyperactive) versus ADHD (Combined) or ADHD (inattentive) (Willcutt et al., 2012).

Conclusion:

Plasma BDNF level is higher in patients with ADHD than in normal control further increase in BDNF level occurs with pharmacological treatment

Limitation Of The Study:

Different ranges of plasma BDNF of levels have previously been reported in healthy subjects, and these differences are most likely due to different assay methods used, such as the R& D ELISA kit or the Promega BDNF kit. Different ELISA methods or different types of sampling tubes might lead to differences in measured BDNF levels.

Recommendations:

Further studies are required to determine the source and role of circulating BDNF in ADHD. Future studies are needed to establish the most reliable, accurate method for measurement of BDNF and to determine which source of BDNF platelets, plasma, serum, or whole blood provides the most reliable biological marker of ADHD. Larger sample size should be used to identify whether BDNF level can be used as a marker for ADHD.

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