Correlates of brain derived neurotrophic factor in children with attention deficit hyperactivity disorder: A case-control study

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Background
Brain derived neurotrophic factor (BDNF) appears to intervene in the pathogenesis and treatment response in Attention deficit hyperactivity disorder (ADHD), based on the conceptualization of ADHD as a neurodevelopmental disorder and the importance of the BDNF for normal neural development.
Aim
To estimate the difference in the serum level of BDNF in children diagnosed with ADHD and normal control, find its clinical correlates and to search which factors could predict abnormal level of BDNF.
Subjects and methods
A case control study was done on 35 child newly diagnosed untreated ADHD, control group of 30 healthy children. All were subjected to IQ test (Stanford-Binet), Conner's test to assess severity of different symptoms. Blood sample to determine the level of BDNF.
Results
Serum level BDNF was significantly higher in children with ADHD (0.1596±0.0909 ngm). BDNF was positively correlated with cognitive problems (r=0.345) and negatively correlated with age and IQ (r=–0.399, –0.383 respectively). Predictors for high level of BDNF were age (beta=–0.368), IQ (beta=–0.368) and inattention (beta=0.422).
Conclusion
High serum level of BDNF in children with ADHD could have a role in the etiology of ADHD, affecting cognition and intelligence. The presence of inattention and low intelligence can predict high level of BDNF.

Keywords:
ADHD, BDNF, Egypt, intelligence, predictors

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Introduction

ADHD is considered as a neurodevelopmental disorder which is characterized by severe and age-inappropriate levels of inattention and hyperactivity/impulsivity that are present in at least two areas of life for over 6 months (, 2013). Though after decades of research, the causes of ADHD are still unknown.

Data from neuroimaging, neurochemical and genetics studies in patients with ADHD have evidenced both functional and structural alterations in development in different brain areas (Cortese, 2012). Molecules implicated in neuroplasticity at these areas may potentially have a role in the pathogenetic mechanisms.

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that plays a key role in the regulation of neurogenesis and differentiation of neural pathways during neurodevelopment. Also, it has a role in the modulation of synaptic plasticity and dendritic growth in the adult brain (Vicario-Abejon *et al.*, 2002; Chapleau and Pozzo-Miller, 2007).

BDNF is expressed during periods critical for cognitive development where the expression of BDNF differs by brain regions. Highest BDNF expression in the temporal cortex occurs in infancy, and decreases with age, whereas in young adulthood peak BDNF expression in frontal cortex occur. BDNF expression in the hippocampus remains relatively constant over the lifespan (Webster *et al.*, 2006). Frontal brain regions develop more slowly than other regions, which have protracted developmental trajectories in the frontal cortex which parallel those of children's cognitive and academic abilities (Kaja *et al.*, 2016).

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Mature BDNF is initially synthesized as a precursor protein Pro-BDNF which is converted to mature BDNF by extracellular proteases. Mature BDNF is biologically active. Both proBDNF and mature BDNF play important roles in several physiological functions of neurons, might be related to the pathology of psychiatric disorders as major depression (Jiang and Salton, 2013), schizophrenia (Martinotti *et al.*, 2012), bipolar disorder (Fernandes *et al.*, 2011) and obsessive–compulsive disorder (Maina *et al.*, 2010). Similarly, also in childhood neurodevelopment disorders such as autism (Katoh-Semba *et al.*, 2007) and ADHD.

BDNF appears to intervene in the pathogenesis and treatment response in Attention deficit hyperactivity disorder (ADHD), based on the conceptualization of ADHD as a neurodevelopmental disorder and the importance of the BDNF for normal neural development. In addition, in experimental models, psycho-stimulants and antidepressants increase the brain concentration of BDNF (Carballo *et al.*, 2012).

BDNF is found in both human serum and plasma, where platelets contain large amount of BDNF (Fujimura *et al.*, 2002). Serum levels of BDNF have been found to be 200-fold higher than plasma levels (Rosenfeld *et al.*, 1995). Therefore, the difference between serum and plasma levels of BDNF could reflect the amount of BDNF stored in circulating platelets.

Because of the discrepancies in different studies between plasma and serum level of BDNF in children with ADHD, we aimed at estimating the difference in the serum level of BDNF in children diagnosed with ADHD and normal control, find its clinical correlates and to search which factors could predict abnormal level of BDNF.

Subjects and methods

This is a case-control study that was carried from May 2016 to September 2016. A convenient sample of 65 children 35 with ADHD and 30 control – groups were enrolled. ADHD children were recruited from outpatient clinic of el-zahraa Hospital. Inclusion criteria were: Children newly diagnosed with ADHD before starting treatment, both sexes, age from 5–13 years. Exclusion criteria were: neurological disorders, ASD, IQ below 70, other comorbidity.

All children were subjected to: a- complete physical examination.

b- Psychometric assessment for diagnosing ADHD using structured clinical interview for assessments of psychiatric disorders in childhood according to DSM IV Criteria (Hien *et al.*, 2004).

Intelligence quotient (IQ) using Stanford Binet Intelligence test which is a standardized test that assesses IQ and cognitive abilities in children and adults aged 2 to 23. It tests four areas of intelligence; verbal reasoning, quantitative reasoning, abstract and visual reasoning and short-term memory skills (Terman and Merrill, 1960). Arabic version was used (Melika, 1998).

The symptomatology of ADHD were assessed using Conner's Parent Rating Scale-revised, long version (Conners, 1989). The Arabic version was used in this study which was translated and validated through previous research conducted by Al-Behairy and Aglaan (2009).

Control-group participants were recruited from Pediatric Department of el-zahraa hospital who were matched in age, sex, residency, socioeconomic class and body mass index. They were selected free from any developmental disease or exhibiting serious behavioral problems as determined through parent and teacher history. Parents or Guardians provided written informed consent according to ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Children or adolescents provided verbal assent regarding participation in this study.

Laboratory assessment

Blood sample collection

For ADHD patients and control group, 3 ml blood samples were collected from the participant's antecubital veins in a plain tube. All samples were allowed to clot for 2 hours at room temperature then centrifuged for 15 minutes at 1000×g samples were clear. The supernatant was collected and stored at –20 0C until assays of serum Human BDNF which was assayed using ELISA Human BDNF. ELISA KIT were obtained from Elab science Catalog No:E-e-H0010.

This ELISA kit uses Sandwich-ELISA as the method. The micro ELISA plate provided in this kit was pre-coated with an antibody specific to Human BDNF. Standards or samples were added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for BDNF and Avidin- Horseradish peroxidase (HRP) conjugate was added to each micro plate well successively and incubated. Free components

Table 1 Difference between patients & control regarding different clinical parameters, BDNF level

	Patients No % 35 (100)	Control No % 30 (100)	T/x^2	*P*
Age	8.1±2.5	8.8±3.3	−0.860	0.397
Gender				
Boys	29 (82.9)	26 (86.7)	0.164	0.686
girls	6 (17.1)	4 (13.3)	0.400	0.527
IQ				
Mean	9.1±95.4	97.2±4.4	−1.038	0.308
Borderline	2 (5.7)			
Dull normal	2 (5.7)			
Normal	31 (88.6)			
ADHD subtypes				
Inattentive type	7 (20)			
hyperactive	19 (54.3)			
mixed	9 (25.7)			
BDNF	0.1596±0.0909	0.0744±0.111	3.218	0.003**

**Highly significant $P<0.01$.

were washed away. The substrate solution was added to each well. Only those wells that contain Human BDNF, biotinylated detection antibody and Avidin-HRP conjugate had appeared blue in color. The enzyme- substrate reaction was terminated by adding of a sulphuric acid solution and the color turns yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm +_2 nm.The OD value was proportional to the concentration of BDNF. For calculation of BDNF concentration, the OD of the sample was compared to the standard curve.

Statistical analysis

Data were entered into the SPSS for Windows (version 15.0; SPSS Inc., Chicago, IL, USA) and were analyzed using descriptive and analytic analyses including frequencies, mean and standard deviations, percent, *t*-test, and chi-square test for comparison of quantitative and qualitative variables, respectively, F test for comparison of 3 groups. Pearson correlation coefficients, stepwise regression analysis was done to detect predictors. A *P* value of ≤0.05 was considered significant in all analyses.

Results

Table 1 demonstrate that both patients and control groups were matched regarding age, gender and mean IQ. More than half of the patient group were hyperactive, 20% were inattentive. The level of BDNF was higher in the patient group (mean=0.1596±0.0909 ngm) and was statistically significant different from control (Table 2).

BDNF was positively correlated with cognitive problems (r=0.345) and negatively correlated with age and IQ (r=−0.399, −0.383 respectively) (Table 3).

Table 2 Correlation between BDNF, different psychological, psychometric parameters

Psychological parameters	R	*P*
IQ	−0.383	0.031*
Age	−0.399	0.018*
Conner's test		
Cognitive problems	0.345	0.043*
Hyperactivity	0.317	0.064
Inattention	0.254	0.141
Liability	0.180	0.300
Hyperactivity- impulsivity	0.185	0.289

*Significant at $P<0.05$.

On comparing between different subtypes of ADHD, there weren't any significant difference between them regarding IO and BDNF level (P=0.310, P=0.116 respectively). Highly significant statistical differences were observed in the subscales of Conner's test as cognitive problems, hyperactivity, inattention and hyperactivity-impulsivity (P=0.001) (Table 4).

Predictors for high level of BDNF were age (beta=−0.368), IQ (beta=−0.368) and inattention (beta=0.422).

Discussion

In this study we aimed to investigate the difference in the level of BDNF between children with ADHD and normal control, also to define its relation to different clinical features. There was significant statistical difference between patients and control regarding the level of BDNF. The increased BDNF could reflect a compensatory mechanism in the response of abnormal and late brain maturation. This was supported by a previous studies) Shim *et al.*, 2008; Reda *et al.*, 2016; Yeom *et al.*, 2016). Others don't find any difference as in studies of Scassellati *et al.* (2014), Sayyah (2009). This difference could be related to

Table 3 Comparison between different subtypes of ADHD using analysis of variance

	Inattentive type Mean±S.D.	Hyperactive type	Mixed type	f	*P*
IQ	96±7.2	97.8±7.4	93.1±12	1.220	0.310
BDNF	0.1074±0.0414	0.1818±0.105	0.1429±0.018	2.306	0.116
Cognitive problems	79±3.6	70.5±6.9	84.2±3.2	20.773	0.001**
Hyperactivity	44.1±3.9	85.5±9.8	81.6±1.8	664.08	0.001**
Inattentive	76.3±4.5	45±7.3	71.9±4.3	189.49	0.001**
Liability	49.3±1.9	60.7±8.8	65.1±8.9	8.43	0.001**
Hyperactivity-impulsivity	55±13.8	73.6±8.5	68.8±4.3	11.18	0.001**

**Highly significant at $P<0.01$.

Table 4 Predictors of elevated BDNF in children with ADHD

	Adjusted r square	Beta	*P*
Age		−0.368	0.015
IQ	0.385	−0.467	0.004
Inattention		0.422	0.007

Dependent factor: BDNF.

different sampling method, or some possible confounding factors in BDNF measures, such as motor activity, exercise or diet.

In our study BDNF level was correlated negatively with age and it increases in younger age group and was found to be a predictor of raised BDNF level. This was supported by the study of Katoh-Semba *et al.* (2007) who have reported that there is increased serum BDNF level over the first several years and, then decreases after reaching adult levels in humans. Also, by a study made by Corominas-Roso *et al.* (2013) who reported that adults with ADHD (mean age: 33.43±8.99 years) have lower BDNF levels than control adults.

BDNF was found to be negatively correlated with total intelligence and low intelligence was found to be a predictor of high BDNF. This was supported by previous studies of Yeom *et al.* (2016), Sayyah (2009) and Taurines *et al.* (2014), who reported that BDNF was correlated with total IQ and verbal IQ but not with performance IQ. They suggested that dysregulated BDNF may play a role in the development of intellectual disability and found that it can be used as an early biomarker for identification of intellectual disability. Elevated BDNF levels may reflect an abnormal state in prenatal or early postnatal neuronal development.

High level of BDNF was correlated with cognitive problems as detected by a subtest of Conner's scale which was supported by the study of Sayyah (2009) and Yeom *et al.* (2016). Altered cognition together with affected intelligence could reflect a relationship between a polymorphism of the BDNF gene and cognitive functions in humans which was found in studies of Egan *et al.* (2003), Hariri *et al.* (2003) and Lang *et al.* (2009). The latter reported that the Val66Met polymorphism of the BDNF gene, valine (Val) to methionine (Met) substitution at codon 66, is associated with poor episodic memory, abnormal hippocampal activation, abnormal intracellular trafficking and dys-regulation of BDNF secretion. This result coincides with a study with Yeom *et al.* (2016), who found similar results in normal preschool children as it is thought that excess BDNF may interfere with normal learning and memory. Also with a study of Vyas and Puri (2012) who reported that BDNF genotype may play a role in cognitive functions and different dimensions of intelligence.

No significant difference between various subtypes of ADHD regarding IQ and BDNF while there were significant differences regarding various sub items of Conner's scale according to the diagnostic subtype.

From the predictors of raised level of BDNF is inattention. This was supported by previous studies of Tsai (2007) and Sayyah (2009), who found a correlation between severity of inattention and raised BDNF. As it was supposed that adequate, physiological amount of BDNF is essential for learning and memory while either increased or decreased level of BDNF will lead to loss of synaptic refinement with impaired learning and memory (Cunha *et al.*, 2010).

Conclusions & recommendation

BDNF level is high in the serum of children with ADHD. BDNF correlated positively with cognitive problems, negatively with age and IQ. Predictors for elevated BDNF are lower age, low IQ and presence of inattention.

Future studies are needed for cognitive, neuro-psychological and psychopathological assessment in a larger sample could be useful to clarify the involvement of BDNF in the phenotype characterization of ADHD. Further studies are required to determine the source of BDNF whether platelet, plasma, serum or whole blood.

Limitations

This study is cross-sectional done at one setting, thus, a more longitudinal studies are needed to determine which variables could have an effect on the level of BDNF. The influence of motor activity, diet and exercise on BDNF weren't considered in this study. Also, small number of the sample, including higher number of subjects would increase statistical power. This would limit the generalizability of the results.

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Conflicts of interest

There are no conflicts of interest.

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