Research Article

Effect of treatment by atomoxetine on the serum level of brain derived neurotrophic factor in children with Attention Deficit Hyperactivity Disorder

Mariam R. Younan*, Samira Z. Said*, Samir M. Abd Alkarim, and Waleed M. Abd Alwahab***

* Department of Pediatric, El-Minia Faculty of Medicine

** Department of Clinical Pathology, El-Minia Faculty of Medicine

Abstract

Objective: To assess the effect of treatment by Atomoxetine (ATX) on the serum level of Brain derived neurotrophic factor (BDNF) in children suffering from Attention deficit hyperactivity (ADHD) treated by ATX in Obstetrics, Gynecology and Pediatric Minia University Hospital, Minia city, Minia Governorate. **Method:** we studied 60 children who were subjected to the following tool; laboratory workup for serum level of BDNF. **Results:** The study revealed that there was significant decrease in serum BDNF level in ADHD children treated by ATX than in ADHD without medications, no significant difference in serum BDNF level between both patients and control groups. There was no difference between serum BDNF level and duration of treatment by ATX in children treated by it. There was highly significant negative correlation between serum BDNF level and Body mass index (BMI) in both patient groups. There was a highly significant negative correlation between serum BDNF level and age in patients groups. **Conclusion:**A significant decrease in serum BDNF level in ADHD children treated by ATX than in ADHD without medications, no significant difference in serum BDNF level between both patients and control groups. There was no difference between serum BDNF level and duration of treatment in group I. The majority of our results are to some extent matched with the literature of ADHD while some results aren't the same, which may be attributed to cultural characteristics and socio demographic issues.

Key Words: Adhd, Bdnf, Atx.

Introduction

Attention deficit hyperactivity disorder (ADHD) is the most common chronic disorder, which starts in early childhood, it is characterized by development with an inappropriate levels of inattention, hyper-activity, and impulsivity that result in impaired functioning across important domains of daily life (American psy-chiatric association., 2013), ADHD should be considered in children with poor attention, distractibility, hyperactivity, impulsiveness, poor academic performance, or behavioral problems at home and school (Christener et al., 2013). The prevalence of ADHD in Minia City (Egypt) is 6.5%, and the prevalence rates of different subtypes in order of frequency are; hyperactive impulsive type (3.51%), then combined type (2.13%), and lastly inattentive type (0.86%), the prevalence in males is (4.01%), while in females (2.48%) with male to female ratio is 1.67:1 (Soliman et al., 2010).

The initial ADHD hypotheses of reduced brain function were based on several obser-vations of reduced volume or functionality of gray and white matter in the brain, leading to deficits in cognitive processing, attention, motor planning, speed of processing responses, and other behavioral issues (Cortese., 2012), The etiology of ADHD is considered a complex of both genetic and environmental factors having key roles (Thapar et al., 2013).

The diagnosis of ADHD is based on criteria specified by the Diagnostic and statistical manual of mental disorders 5th edition, there

are predominant three subtypes of ADHD: inattentive, hyper-activity–impulsive and combined subtypes (APA, 2013), the management of ADHD depends on behavioral therapy, dietary modifications, medications including stimulant and nonstimulant drugs (christner et al., 2013).

Brain-derived neurotrophic factor (BDNF) is a member of neurotrophin family (Quintero et al., 2013). BDNF modulates dendrites complexity and spine density, which markedly affects behavior and suggests that it acts more as a differentiation and plasticity factor in the central nervous system (Agustin et al., 2014).

It is suggested that neuronal spine formation and plasticity might underlie the pathophysiology of ADHD (Lesch et al., 2008).

Exercise may improve executive functionning and behavioral symptoms associated with ADHD, and BDNF was involved in mediating these effects (Rommel et al., 2013).

Experimental data from animal models provide a proof that BDNF plays a role in the mechanism of action of ATX, treatment by it increased BDNF mRNA levels in the hippocampus and prefrontal cortex and frontal cortex of spontaneously hypertensive rats (Fumagalli et al., 2010). Another study reported a decrease in BDNF level after ATX treatment in inattentive subgroup of ADHD (Corominas et al., 2013).

Atomoxetine is the first non stimulant drug to be approved by FDA treatment of ADHD (Childress., 2016). The Mechanism by which ATX acts is unclear (Karly et al., 2009), but is expected to be related to its selective inhibition of presynaptic NE reuptake (Dopheide et al., 2009). ATX primary advantage over the standard stimulant treatments for ADHD is that it has little known abuse potential (Ghuman et al., 2014).

There is an alarm from Food and drug association boxed warning for ATX because it has been associated with an increased risk of suicidal thinking in children and adolescents with ADHD, although ATX has been associated with less growth suppression compared with stimulants, growth should be monitored during treatment as height and weight gain may be reduced during the first 9 to 12 months of treatment, but should recover by 3 years of therapy (Dopheide et al., 2009).

This study aimed to assess the effect of treatment by ATX on the serum level of BDNF in ADHD children.

Subjects and Methods

The present study is a case control study assessing the effect of treatment by ATX on the serum level of BDNF in ADHD children, the study included 60 children with different ages and sex from Obstetrics, gynecology and children Minia University Hospital in Minia city, Minia governorate, Egypt. The study was conducted from September 2016 to July 2017.

The ethical guidelines of the research were followed. The study was conducted in two stages.

Stage I: Blood sample collection**,** blood samples were taken and evacuated into serum separator tube and samples allowed to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 × g, then serum removed and put into aliquot and samples stored at -20℃ .

Stage II: Measurement of serum BDNF, the microtiter plate has been pre-coated with an antibody specific to BDNF, Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for BDNF and Avidin conjugated to Horseradish Peroxidase was added to each microplate well and incubated, then a substrate solution is added to each well. Only those wells that contain BDNF, biotin-conjugated antibody and enzyme-conjugated Avidin exhibit a change in color, the enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change was measured spectro photometrically at a wavelength of 450 nm ± 2 nm, the

concentration of BDNF in the samples is then determined by comparing the samples to the standard curve.

There were three groups in the study:
1- 20 children with ADHD treated by ATX for 9 month duration.
2- 20 children with ADHD before start of any medical treatment.
3- 20 healthy children not experience ADHD previously which serve as a control group.

Statistical analysis

Data obtained from our study were fed into an IBM compatible computer. Data were coded, entered and analyzed. Descriptive statistics (mean (M), standard deviation (SD), frequencies and percentages) were calculated using a computer software package (SPSS, version 10) and analysis was performed using the student's T-test, Chi-square X2 and Z test. Analysis of variants (ANOVA) was applied to compare mean values of more than three groups, P values <0.000 were considered statistically significant.

Results

Our results can be demonstrated in the following tables:

Table (1): shows that the age at data collection was 8.81± 2.49 years in patients group and 8.05 ± 2.03 in control group, the proportion of males was 100 % in patients group and 50% in control group, the mean of BMI was 16.04± 3.00 in patients group and18.21 ± 2.50 in control group, the mean BDNF was13.09±8.91 in patients group and 14.81+4.05 in control group,no differences in mean age ($p = 0.24$) in both groups, mean BMI highly significant correlation between both groups ($p = 0.007$), sex highly significant correlation between both groups (p=0.000), no differences in mean BDNF level (p=.41) in both groups

Table (2) shows that the mean age was 10.67±2.10 in group I, 6.95±1.02 in group II, the mean weight was 37.47±10.37 in group I and 20.22±3.63 in group II, the mean height was142.3±12.73 in group I and 119.4±8.29 in group II, the mean BMI was 18.03 ± 2.85 in group I and 14.05± 1.41 in group II, the mean BDNF was 6.11±4.70 in group I and 20.07±6.18 in group II, there were highly significant correlations with (p=0.000) between the mean age, weight, height, BMI, BDNF level in both groups and no significant difference between types of ADHD in both groups with (p=0.28).

Table (3) shows that the mean age was 6.95±1.02 in group II and 8.05±2.03 in group III, the mean weight was 20.22±3.63 in group II and 29.70± 9.04 in group III, the mean height was 119.4±8.29 in group II & 126.4±11.4 in group III, the mean BMI was 14.05±1.41 in group II & 18.21± 2.50 in group III, the mean BDNF level was 20.07±6.18 in group II and 14.81±4.05 in group III, there was no difference between mean age (p=0.037) in both groups, there was highly significant correlation between mean weight (p=0.000)in both groups, there was no difference between mean height (p=0.032) in both groups, there was highly significant correlation between mean BMI (P=0.000) in both groups, there was highly significant correlation between mean BDNF level (p=0.003) in both groups, and there was no difference between gender in both groups.

Table (4) shows that there was highly significant negative correlation between BDNF level and age (p=0.000) in both patient groups, there was highly significant negative correlation between BDNF level and BMI (p=0.000) in both patient groups, there was no difference between BDNF level and duration of treatment in group I, there was no difference between BDNF level and ADHD types in both patients groups.

Table (1): Demographic data for all cases in this study.

Variables	Control Groups (n = 20)		Patients Groups (n = 40)		P-value
	Range	Mean ± SD	Range	Mean ± SD	
Age (Years)	6 – 14	8.05 ± 2.03	5.5–14	8.81± 2.49	0.24
Weight (kg)	19 – 50	29.70 ± 9.04	14–52	28.85+11.6	0.77
Height (cm)	112-157	126.4±11.4	32– 40	130.8±15.7	0.26
BMI	14.90–22.30	18.21 ± 2.50	11.8-21.7	16.04± 3.00	0.007**
Sex (male/female)	10 (50%) / 10 (50%)		40 (100%) / 0 (0%)		0.000**
BDNF level (ng/ml)	8.10 – 21.70	14.81+4.05	2.2-30.2	13.09±8.91	0.41

Table (2): Comparison between group I and Group II in patients groups.

Variables	Group I (ADHD treated by ATX) (n = 20)		Group II (ADHD) (n = 20)		P-value GI vs GII
	Range	Mean ± SD	Range	Mean ± SD	
Age (Years)	6 –14	10.67 ± 2.10	5.5 – 9	6.95 ± 1.02	0.000**
Weight (kg)	16–52	37.47±10.37	14 – 28	20.22±3.63	0.000**
Height (cm)	112– 157	142.3±12.73	105-137	119.4±8.29	0.000**
BMI	12.7-21.7	18.03 ± 2.85	11.8–17.8	14.05± 1.41	0.000**
BDNF level	2.2-20.4	6.11±4.70	9.4–30.2	20.07±6.18	0.000**
ADHD type: **Combined** **Inattentive** **Hyperactive**	 16 (80%) 3 (15%) 1 (5%)		 13 (65%) 2 (10%) 5 (25%)		 0.28

Table (3): Comparison between group II and Group III in the study.

Variables	Group II (ADHD) (n = 20)		Group III (Control) (n = 20)		P-value GiIvs GIII
	Range	Mean + SD	Range	Mean + SD	
Age (Years)	5.5 – 9	6.95 + 1.02	6 – 14	8.05 + 2.03	0.037*
Weight (kg)	14 – 28	20.22+3.63	19 – 50	29.70+ 9.04	0.000**
Height (cm)	105-137	119.4+8.29	112-157	126.4+11.4	0.032*
BMI	11.8–17.8	14.05+ 1.41	14.9–22.3	18.21+ 2.50	0.000**
BDNF level	9.4–30.2	20.07+6.18	8.1–21.70	14.81+4.05	0.003**
Sex (male/female)	20 (100%) / 0 (0%)		10 (50%) / 10 (50%)		1.00

Table (4): Correlation between BDNF and age, BMI, duration of treatment by ATX, type of ADHD for patients groups (n=40).

Variables	Correlation (r)	P-value (P)
BDNF level and age	-0.684	0.000**
BDNF level and BMI	-0.652	0.000**
BDNF level and duration of treatment by ATX	-0.148	0.534
BDNF level and ADHD type	0.279	0.081

Discussion

Our work is a case control study that was conducted on 60 children were attending pediatric neurology clinic at Obstetrics, gynecology and children Minia University Hospital, Minia city, Minia Governorate: (20 children with ADHD who were treated by ATX, 20 children with ADHD were not on medical treatment), and 20 children as control group.

AS shown in table (1) in the regard of sex and BMI there was highly significant correlation between patients and control groups. In contrast our study disagree with Scassellati et al., 2014 who found similar BMI values for both the ADHD subtypes and the controls .Also no differences in serum BDNF level in both patients and control groups. Our finding were in agreement with Aynur Pekcanlar et al., 2017 who reported that fifty patients and fifty healthy controls were enrolled in their study, there was no statistically significant difference between boys with ADHD and healthy controls in terms of baseline serum BDNF levels. Similarly Seref et al., 2016 reported that one of the most important findings in their study is that there was no difference in BDNF levels between the ADHD and control groups. However, Shim et al., 2008 reported significantly higher BDNF levels in ADHD children when compared to controls.

Table (2) showed that there were highly significant correlations between the mean age, weight, height, BMI in both patients groups, and decreased serum BDNF level in group I than in group II. Our results supported by Karly et al., 2009 who reported that ATX was associated with an initial loss in expected height and weight among ATX recipients, this eventually returned to normal in the longer term. Another study done by Kratochvil et al., 2014 as regard to the safety extrapolation analyses, decreased appetite was the most common in ATX treated patients in both 6–7-year-old patients and 5-year-old patients, and it was observed with a higher incidence in 5-year-old patients.

Also in our study as shown in table (3) there was highly significant correlation between age in both group II &group III, there was significant correlation between mean weight in both groups, there was highly significant correlation between height in both groups, there was no significant difference between BMI in both groups, there was highly significant correlation between BDNF level increased in untreated patient group more than control group and there was no difference in gender in both groups.

Shim et al., 2008 who found that children (mean age: 8.8 ± 2.3 years), who are diagnosed with ADHD, have higher plasma BDNF levels than control children, and the severity of inattention problems have a positive correlation with plasma BDNF levels and they suggested that increased BDNF levels possibly reflect a compensatory mechanism in the response of abnormal and late brain maturation. However Catia et al., 2014 said that their findings indicate no alteration of serum BDNF levels in untreated patients with ADHD. As regard BMI Holtkamp et al., 2004 evaluated a sample of 97 boys with ADHD in Germany to test the hypothesis that hyperactive boys would have a lower prevalence of obesity than an age-matched healthy male reference population, contrary to expectations, they found that a significant number of subjects with ADHD had a BMI $\geq$ 90th percentile (19.6%) and 7.2% had a BMI $\geq$ 97th percentile using the higher International Obesity Task Force cut-offs points.

Table (4) shows that there was highly significant negative correlation between BDNF level and age in both patient groups, there was highly significant negative correlation between BDNF level and BMI in both patient groups, there was no difference between BDNF level and duration of treatment in group I, there was no difference between BDNF level and ADHD subtypes in both patients groups. As regard relation between serum level of BDNF and duration of treatment by ATX a study done by Cubero-Millán et al., 2017 suggested that the magnitude of the difference in serum BDNF levels between patients and controls was much greater than the change in serum BDNF after chronic treatment

with ATX. In literature, another study done by Josep et al., 2014 was in accordance to our work in their first study to assess the effects of ATX treatment on serum BDNF levels in with ADHD, these patients received ATX for 3 months, at baseline, there were no differences between the two subgroup of treated patients (completed/ discontinued treatment) in terms of their serum BDNF levels. Scassellati et al., 2014 their study revealed that no difference in BDNF levels was reported between disease subtypes.

References

1. Agustin A and Barbara LH: BDNF function in health and disease, nature review sneuroscience, EVER Neuro Pharma GmbH, Oberburgau Unterach, Austria 2014;15:(2).
2. American Psychiatric Association: Diagnostic and statistical manual of mental disorders (5th ed.) Washington, DC: American Psychiatric Association. 2013.
3. Aynur Pekcanlar Akay, Halil Resmi, Sevay Alsen Guney, Handan Ozek Erkuran, Gonca Ozyurt, Enis Sargin, Ahmet Topuzoglu, and Ali Evren Tufan: Serum brain-derived neuro trophic factor level sin treatment aïve boys with attention deficit/hype
4. Activity disorder treated with methyl phenidate: an 8-week, observational pretest–post test study 2017; 27(1): 127 –135.
5. Catia Scassellati, Roberta Zanardini, Alessandra Tiberti, Marco Pezzani, era Valenti, Paola Effedri, Elena Filippini, Stefano Conte, Alberto Ottolini, Massimo Gennarelli, Luisella Bocchio-Chiavetto: Serumbrain derived neurotrophic factor (BDNF) levels in attention deficit disorder European Child & Adolescent Psychiatry, 2014; 23; (3): 173–177.
6. Childress AC: A critical appraisal of atomoxetine in the management of ADHD. Ther Clin Risk Manag, 2016; (12):27–39.
7. Christner J, O'Brien JM, Felt BT, Harrison RV, Kochhar PK, Bierman B: Attention-deficit hyperactivity disorder. Ann Arbor, Mich.: University of Michigan Health System; 2013.
8. Cortese S, and Vincenzi B: Obesity and ADHD: Clinical and Neurobiological Implications. Curr Top Behav Neurosci 2012; 9:199–218.
9. Corominas-Roso M, Ramos-Quiroga JA, Ribases M, Sanchez-Mora C, Palomar G, Valero S, Bosch R, Casas M: Decreased serum levels of brain-derived neurotrophic factor in adults with attention deficit hyper activity disorder. Int J Neuropsycho pharmacol 2013, 16(6):1267-1275.
10. Cubero-Millán, I., Ruiz-Ramos, MJ., Molina Carballo: BDNF concntrations and daily fluctuations differ among ADHD children and respond differently to methyl ephenidate with no relationship with depressive symptomatolgy. Psycho pharmacology, 2017; 234 (2): 267-279.
11. Dopheide JA, Pliszka SR. Attention-deficit-hyperactivity disorder: Attention deficit hyperactivity disorder, anupdate. Pharmacotherapy, 2009; 29(6):656-79.
12. Fumagalli F, Cattaneo A, Caffino L, Ibba M, Racagni G, Carboni E, Gennarelli M, Riva MA: Sub-chronic exposure to atomoxetine up-regulates BDNF expression and signalling in the brain of adolescent spontaneously hypertensive rats: comparison with methylphenidate. Pharmacol Res, 2010: (62)523–529.
13. Ghuman JK, Hutchison SL: Atomoxetine is a second-line medication treatment option for ADHD. Evid Based Ment Health, 2014; 17(4): 108.
14. Holtkamp K, Konrad K, Muller B, Heussen N, Herpertz S, Herpertz-Dahlmann B, Hebebrand J: Overweight and obesity in children with attention deficit/hyperactivity disorder. International Journal of Obesity and Related Metabolic Disorders.2004,28(5):685-89
15. Josep Antoni Ramos Quiroga, Margarida Corominas Roso, Gloria Palomar, Nuria Gomez Barros, Marta Ribases, Cristina Sanche, Mora, Rosa Bosch, Mariana Nogueira, Montserrat Corrales, Sergi Valero, Miguel Casas: Changes in the serum levels of brain-derived neurotrophic factor in adults with attention deficit hyperactivity

disorder after treatment with atomoxetine, 2014; Vol. 231:(7).
16. Karly PG, Gillian MK: Atomoxetine a review of its use in Attention-Deficit Hyperactivity Disorder in children and adolescents. Paediatric drugs, 2009; 11: 203-226.
17. Kratochivl C, Chumam J, Camporeal A, Lipsius S, D Souzae, Tanaka Y: Efficacy and Safety Extra polation Analyses for Atomoxetine in Young Children with Attention Deficit/ Hyperactivity Disorder 2014; 25:(10).
18. Lesch KP, Timmesfeld N, Renner TJ: Molecular genetics of adult ADHD converging evidence from genome-wide association and extended pedigree linkage studies. J Neural Transmembr, 2008; 115(11):1573–1585.
19. Nelson KB, Grether JK, Croen LA, Dambrosia JM, Dickens BF, Jelliffe LL, Hansen RL, Phillips TM: Neuropeptides and neurotrophins in neonatal blood of children with autism or mental retardation. Ann Neurol, 2001; 49: 597–606.
20. Quintero GC: Role of nucleus accumbens glutamatergic plasticity in drug addiction. Neuropsychiatr Dis Treat, 2013; 9:1499–1512.
21. Rommel AS, Halperin JM, Mill J: Protection from genetic diathesis in attention-deficit/hyperactivity disorder: possible complementary roles of exercise. J Am Acad Child Adolesc Psychiatry, 2013; 52(9):900–910.
22. Seref Simsek, Salih Genconglan, TugbaYskel, Ibrahim Kaplan, Huseyin Aktas and Rumeysa Alaca: Evaluation of the Relationship between Brain-Derived Neurotropic Factor Levels and the Stroop Interference Effect in Children with Attention-Deficit Hyperactivity Disorder. Noro Psikiyatr Ars 2016; 53(4): 348–352.
23. ShimSH, Hwangbo Y, Kwon YJ, Jeong HY, Lee BH, Lee HJ, Kim YK: Increased levels of plasma brain-derived neurotrophic factor (BDNF) in children with attention deficit hyperactivity disorder (ADHD). Prog Neuropsycho pharmacol Biol Psychiatry, 2008; 32:1824-1828
24. Soliman TG, Mohamed F. Afify, Mohamed A. Yehia, Emad A. Abdel-Naem and Samir M. Abd Alkarim: Attention deficit hyperactivity disorder "Anepidemiologic study of preschool and primary school children in Minia city, EL-MINIA MED2, Vol 21:(1).
25. ScassellatiC,Zanardini R, Tiberti A, Pezzani M, Valenti V, Effedri P, Filippini E, Conte S, Ottolini A, Gennarelli M, Bocchio-Chiavetto L: Serum brain-derived neurotrophic factor (BDNF) levels in attention deficit hyperactivity disorder (ADHD). Eur Child Adolesc Psychiatry, 2014; (23): 173–177.
26. Thapar A, Cooper M, Eyre O, Langley K: What have we learnt about the causes of ADHD? J Child Psychol Psychiatry 2013; 54: 3–16.