

SERUM BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF): THE SEVERITY AND SYMPTOMATIC DIMENSIONS OF DEPRESSION

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SUMMARY

Introduction: The aim of this study was to compare the concentration of serum Brain-derived neurotrophic factor (BDNF) in patients suffering from major depressive disorder (MDD) considering the severity of MDD episode defined by the Hamilton rating scale for depression (HAMD-17). The other aim was to research the connection between serum BDNF and the symptomatic dimensions of MDD.

Subjects and methods: The study includes 139 participants with major depressive disorder (MDD). Diagnosis of MDD was set by DSM-IV-TR criteria. The severity of MDD was estimated with HAM-D-17 in the manner that mild episode was diagnosed if the score on HAMD-17 was up to 18, moderately severe 18-25 and severe over 25. Concentration of BDNF was determined by the ELISA method.

Results: This research could not find a difference in BDNF concentration considering the severity of the depressive disorder in groups suffering from mild, moderately severe and severe episodes of MDD ($F=1.816$; $p=0.169$). Factor analysis of HAMD-17 extracted four dimensions of depressive symptoms. None of the symptomatic dimensions was significantly related to BDNF concentration.

Conclusion: Results of this study indicate that serum BDNF levels are not related to the severity of depression and its specific symptomatic dimensions. These findings support the idea of a complex relationship between BDNF concentration at the periphery and in the CNS.

Key words: brain derived neurotrophic factor – BDNF - depression – MDD - biological marker

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INTRODUCTION

Brain-derived neurotrophic factor (BDNF) was discovered as the second member of the neurotrophic factors family that showed certain influence on the survival of the subpopulation of neuron dorsal ganglia (Barde et al. 1982). After that, other members of neurotrophic family were discovered: neurotrophin-3 (NT-3) (Maisonpierre et al. 1990) and neurotrophin 4/5 (NT-4/5) (Hallbook et al. 1991), each with a different profile of trophic effects on a subpopulation of neurons at the periphery and in the CNS. BDNF is a 27-kDa polypeptide with a major role in survival, differentiation and growth of selective central neurons during childhood and adult age (Huang et al. 2001, Jakovljević, 2011). Primarily isolated as a secretory protein that encourages survival of peripheral neurons, BDNF was also recognised as a significant factor in regulating a major range of functions, including survival of neurons, migration, phenotypic differentiation, growth of axons and dendrites and synapse formations (Hallbook et al. 1991, Huang et al. 2001). Also, it has a key role in neuroplasticity in several brain areas (McAllister et al. 1999, Poo 2001, Lu et al. 2003). The latest research

emphasises the role of BDNF in cognitive functioning, especially memory, which is directly linked to neurobiology of depressive disorder (Tyler et al. 2002, Pang et al. 2004, Lu et al. 2006).

Due to difficulties related to measuring BDNF directly in the brain tissue, there was a major interest in defining concentration of BDNF at the periphery which can be performed non-invasively from blood samples. Karege et al. (2002) were one of the first to find correlation between cortical and serum BDNF on animals. Moreover, strong evidence in the animal model suggest BDNF is involved in pathogenetic mechanism of MDD (Duman & Monteggia 2006). Afterwards, this was followed by studies that defined BDNF concentration in patients suffering from depression. Many recent studies of BDNF found a reduced concentration in depressive patients compared with the healthy control group (Gervasoni et al. 2005, Gonul et al. 2005, Karege et al. 2005, Aydemir et al. 2006, Aydemir et al. 2007, Huang et al. 2007, Yoshimura et al. 2007, Monteleone et al. 2008, Piccinni et al. 2008, Sozeri-Varma et al. 2011). Especially significant are the results of the meta-analysis conducted by Sen et al. (2008) that confirmed previously stated findings of lower BDNF concentration

in the group of patients with MDD in relation to the group of healthy examinees. Also, one even more recent meta-analysis extended the same conclusion to both serum and plasma BDNF levels (Bocchio-Chiavetto et al. 2010). These findings have been put in a broader frame when several studies analyzed BDNF concentration in depressive patients before and after therapy with antidepressants (Karage et al. 2002, Shimizu et al. 2003, Gonul et al. 2004, Yoshimura et al. 2007, Molendijk et al. 2011). Brunoni et al. (2008) conducted the meta-analysis of such studies and showed the increase of BDNF levels after therapy as well as significant positive correlation between changes in BDNF concentration and total scores on depression rating scales.

However, research so far hasn't provided consistent and stable results on the relationship between BDNF concentration and the severity of MDD (Molendijk et al. 2011, Sozeri-Varma et al. 2011). Also, research on BDNF relevance in specific clinical features of depression has been very scarce. So, the aim of this study was to compare the concentration of serum BDNF in patients with the major depressive disorder (MDD) considering the severity of MDD episode (mild, moderately severe and severe clinical picture) and the symptomatic dimensions of depression.

SUBJECTS AND METHODS

Subjects

This study included 139 patients of both sexes, 64 (46%) female and 75 (54%) male, treated for MDD at the Clinic. Those suitable for participation were explained the purpose and the aim of this investigation which was validated by the Ethical Commission of the Clinical Centre.

Research did not include patients with some other comorbid DSM-IV diagnosis, including abuse of psychoactive drugs and alcohol, or any other physical illness (diabetes, arterial hypertension, obesity and other). All examinees abstained from psycho-pharmaceutical therapy for a minimum of two weeks. Other criteria for participation were the following: signed approval of participation in the research; no therapy for reducing blood fat, steroids, thiazide diuretics, anti-coagulants, β -blockers and non-steroidal anti-inflammatories; weight not varying more than $\pm 5\%$ in the three months prior to the study; women were not pregnant nor breastfeeding; between 25 and 70 years of age.

Diagnostic instruments

The study included a structured questionnaire that included socio-demographic anamnestic variables: sex, age, duration of disease, duration of the current episode, total number of episodes of MDD, time period between episodes of illness, start of the first episode.

All patients who participated in our study were diagnosed with MDD. Other psychiatric diagnoses were excluded by MINI (Mini-International Neuropsychiatric Interview questionnaire (Sheehan, Lecruiber & Sheehan 1998) based on DSM-IV criteria. It is a short structured clinical interview which enables diagnoses of psychiatric disorders, time of the interview is approximately 15 minutes and was initially designed for epidemiological studies and multicenter clinical trials (Shvartzman et al. 2005).

Severity of depression was assessed by one of the most commonly used psychiatric depression rating scales – Hamilton rating scale for depression (HAMD17) (Hamilton 1960). Most research show relatively high test-retest and inter-rater reliability with a more varying internal consistency (Bagby et al. 2004). Also, validation studies speak of HMD-17's multidimensionality and even though the number of factors is somewhat unstable, Cole et al. (2004) identified the four factor structure as the most consistent one. This instrument consists of 17 items, each can be scored from 0 to 4 and the highest possible score is 53. In clinical practice it is accepted that in mild depression (F32.0) the HAMD-17 total score does not exceed 18 points, in a moderately severe depressive episode (F32.1) total score is between 18 and 25 points, while in severe depression (F32.2) it does not exceed 25 points (Jevtović et al. 2011).

Laboratory methods for defining BDNF

All examinees had one test tube of blood taken from the cubital vein, 12 hours after their last meal, in the morning (about 8 a.m.), without anti-coagulants in order to define BDNF. Testing did not include hemolytic and lypemic samples. Samples were stored at a temperature of -25°C prior to testing. All laboratory parameters were set three times and the mean value was calculated. All biochemical analyses were performed in the Clinical Institute for Chemistry at the University Hospital Centre „Sestre milosrdnice“. The mentioned laboratory is certified for performing all of the above analyses according to ISO standard HRN EN ISO 15189 (ISO 15189:2006; EN ISO 15189:2003) and monitored through external review.

After the blood coagulates, serum is divided by centrifugation at room temperature at $2000\times g$. The serum is stored at -20°C until the moment of defining the BDNF concentration. BDNF concentration is defined by the ELISA method (Enzyme Immunoassay) of the company RayBiotech, Inc.

RayBio Human BDNF ELISA (Enzyme-Linked Immunosorbent Assay) is a kit for quantitative in vitro definition of BDNF in serum, plasma, cell cultures and urine. This test uses anti-bodies, specific for human BDNF, that are spread on the board with 96 wells. Standards and samples are pipetted into wells and BDNF present in samples is tied with immobilised

antibodies. The wells are rinsed and „biotynylated“ anti-human BDNF antibody is added. After the rinse, untied „biotynylated“ antibody, HRP-conjugated streptavidine is pipetted into the wells. Wells are then rinsed again, TMB substratum solution is added into wells and the colour develops proportionally to the amount of the tied BDNF. Stop solution changes colour from blue to yellow and the intensity of the colour is measured at 450 nm. Minimal concentration of BDNF for detection is commonly lower than 80 pg/ml. The concentration of serum BDNF is expressed in ng/ml.

Statistical methods

Socio-demographic data were analyzed using the nonparametric χ^2 test and are presented by frequencies and percentages. ANOVA was used for parametric data, having demonstrated a normal distribution by the Kolmogorov-Smirnov test. Factorization of the HAMD-17 was conducted using factor analysis with VARIMAX rotation and retention of regression scores of individual factors. These regression scores were used in multiple regression analysis. Statistical significance was set at 5% and all the analyses were performed using the statistical package SPSS 11.

RESULTS

Socio-demographic data, number of hospitalisations and duration of illness, as well as the total scores on

HMD-17 differentiating between the groups of patients with mild, moderate and severe depressive episode are presented in Table 1.

This study did not show any difference in the concentration of serum BDNF considering the severity of depression in groups with mild, moderately severe and severe MDD ($F=1.816$; $p=0.169$). Concentration of serum BDNF in patients with mild form of MDD was (mean \pm SD) 41.7 \pm 14.5, moderately severe symptoms of MDD amounted to 36.4 \pm 14.8, while severe symptoms had a concentration of 42.1 \pm 11.1 (Figure 1).

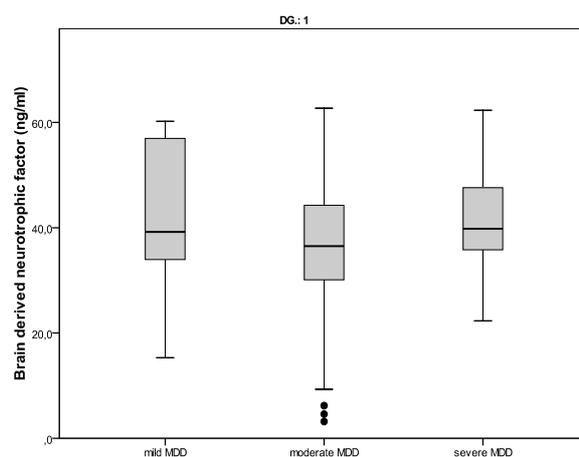


Figure 1. Concentration of serum BDNF (ng/ml) in groups with mild, moderate and severe episode of MDD

Table 1. Socio-demographic data, data on the number of hospitalisations and duration of disorder, Hamilton rating scale totalscore

	Mild	Moderate	Sever	Statistics
Sex: N (%)				
Male	10 (57.9)	36 (53.6)	29 (52.6)	$\chi^2=160$ (0.923)
Female	7 (42.1)	31 (46.4)	26 (47.4)	
Education: N (%)				
Elementary	4 (21.1)	16 (25.4)	5 (8.8)	$\chi^2=13.075$ (0.011)
High school	8 (42.1)	40 (63.5)	43 (75.4)	
University	7 (36.8)	7 (11.1)	9 (15.8)	
Marital status: N (%)				
Single	14 (73.7)	47 (74.6)	41 (71.9)	$\chi^2= 2.530$ (0.639)
Married	3 (15.8)	13 (20.6)	9 (15.8)	
Divorced/widow	2 (10.5)	3 (4.8)	7 (12.3)	
Working status: N (%)				
Unemployed	4 (21.2)	26 (41.3)	28 (49.1)	$\chi^2= 13.046$ (0.011)
Employed	3 (15.8)	18 (28.6)	18 (31.6)	
Retired	12 (63.2)	19 (30.2)	11 (19.3)	
Place of residence: N (%)				
Urban	15 (78.9)	39 (61.9)	35 (39.3)	$\chi^2= 2.130$ (0.345)
Rural	4 (21.1)	24 (38.1)	22 (38.6)	
Age in years (mean \pm SD)	41.96 \pm 13.2		43.2 \pm 12.4	$F=-1.707$; $p=0.187$
No. of hospitalizations (mean \pm SD)	2.07 \pm 2.187	4.38 \pm 3.927	4.08 \pm 1.552	$F=-3.096$; $p=0.50$
Duration of disorder in years (mean \pm SD)	2.33 \pm 0.976	2.31 \pm 1.365	1.76 \pm 0.779	$F=-2.021$; $p=0.139$
Hamilton rating scale score (mean \pm SD)	10.47 \pm 5.540	15.17 \pm 6.552	23.40 \pm 7.879	$F=-19.774$; $p=0.000$

Factor analysis of HAMD-17 rating scale extracted four dimensions of MDD symptoms. They are represented in Factor 1 (items Anxiety (physical) H11, Feelings of guilt H5, Work and activity H7, Difficulties in dreaming H2, General physical symptoms H4, Anxiety (mental) H10 and Difficulties in falling asleep H13), Factor 2 (items Psychomotoric inhibition H8, Gastrointestinal symptoms H12, Depressive mood H1, Morning sleep difficulties H6), Factor 3 (items Genital symptoms H14, Hypochondria symptoms H15, Agitation H9, Insight into illness H17, Suicidal tendencies H3) and Factor 4 (items Weight loss H16a and 16b). The above mentioned dimensions of depression with the according factors are presented in Table 2.

Table 2. Factor analysis of HAMD-17 with four dimensions of MDD symptoms

	Factor
Factor 1	
Hamilton 11	0.823
Hamilton 5	0.718
Hamilton 7	0.639
Hamilton 2	0.612
Hamilton 4	0.604
Hamilton 10	0.556
Hamilton 13	0.534
Factor 2	
Hamilton 8	0.705
Hamilton 12	0.608
Hamilton 1	0.599
Hamilton 6	0.537
Factor 3	
Hamilton 14	0.730
Hamilton 15	0.608
Hamilton 9	0.565
Hamilton 17	0.538
Hamilton 3	0.458
Factor 4	
Hamilton 16b	0.867
Hamilton 16a	0.804

After factorisation of HAMD-17 into four dimensions of symptoms and retaining regression scores for each dimension, they were used in regression analysis. Multiple regression analysis of the connection between BDNF concentration and symptomatic dimensions of

MDD showed no statistically significant model ($F=0.924$; $p=0.454$). None of the HAMD-17 symptomatic dimensions of depression is significantly related to the concentration of serum BDNF (Table 3).

DISCUSSION

In our study, the concentration of serum BDNF was not significantly related to the severity of depressive episodes. In other words, these concentrations were very much alike in all three groups of depressed patients who showed mild, moderate and severe symptoms of MDD, based on their HAMD-17 total scores. More than one meta-analytic investigation confirmed reduction of both BDNF serum and plasma levels in MDD, as well as elevation of these levels following a course of antidepressant treatment (Set et al. 2008, Bocchio-Chiavetto 2010). All these studies speak in favour of the possibility that, especially taken from serum samples, BDNF concentration represents a biologically important factor of MDD and supports the opinion improvement of depressive symptoms could be related to neuroplastic changes as a result of pharmacological therapy. On the other hand, negative correlation of both serum and plasma BDNF levels in relation to the severity of the depressive episode was shown in few studies (Karage et al. 2002, Shimizu et al. 2003, Gonul et al. 2005, Dell'Osso et al. 2010) but this finding wasn't replicated in some other investigations (Lee et al. 2007, Molendijk et al. 2011, Sozeri-Varma et al. 2011), including this one where we showed non-significant differences in serum BDNF concentration between different levels of depression. Since our study and those with similar results had larger non-clinical and clinical samples, we can conclude with a high amount of certainty that other factors, apart from those specifically related to depressive disorder, have a key role in BDNF level in patients suffering from depression. It is known that exercise can affect serum BDNF levels (Gustafsson et al. 2009). Also, three studies have shown stressful life events are related to lower peripheral BDNF levels in healthy groups, depressed and bipolar patients (Kauer-Sant'Anna et al. 2007, Grassi-Oliveira et al. 2008, Mitoma et al. 2008). Bus et al. (2010) recently determined eight independent factors that influence BDNF concentration: time of taking the blood sample, length of sample storage, eating prior to taking the blood sample, place of residence, age, gender, smoking and alcohol.

Table 3. Correlation of HAMD-17 regression score factors with the concentration of serum BDNF

Dependent variable	Predictors	B	SEB	β	t	p	95% CI for B	
							Lower B.	Upper B.
BDNF	Factor 1	2.330	1.741	0.148	1.338	0.184	-1.131	5.791
	Factor 2	-2.373	1.535	-0.169	-1.545	0.126	-5.425	0.679
	Factor 3	-0.508	1.383	-0.040	-0.368	0.714	-3.257	2.240
	Factor 4	0.263	1.367	0.021	0.192	0.848	-2.455	2.981

In addition to these confounding factors, serum and plasma BDNF reduction has been shown in many other psychiatric and neurodegenerative diseases: bipolar disorder (Palomino et al. 2006), schizophrenia (Ikeda et al. 2008), BAP (Machado-Vieira et al. 2007), eating disorders (Nakazato et al. 2003), Huntington's disease (Ciammola et al. 2007), Alzheimer's disease (Yasutake et al. 2006) and autism (Hashimoto et al. 2006). This leads us to think low BDNF levels are not specific to the depressive disorder. In fact, BDNF peripheral concentration might serve as a marker of some underlying neuropathogenetic vulnerability factor that leads to different disorders. Additionally, inconsistent findings on correlation between BDNF levels and severity of depression are linked with the question to what extent BDNF concentration in blood reflects BDNF in the brain. Even though our research used serum BDNF, which has led to more consistent findings across different studies compared with plasma BDNF (Bocchio-Chiavetto et al. 2010), we didn't measure the amount of BDNF in the brain. It is true that cortical and peripheral levels of BDNF are positively correlated (Karege et al. 2002, Klein et al. 2011) but it is still unclear to what extent because other tissues, not only neurons, serve as a source of BDNF in the periphery (Karege et al. 2002, Lommatzsch et al. 2005). In other words, it is still needed to determine if serum BDNF reduction has its origin in decreased BDNF concentration in the brain, peripheral tissues or both (Sen et al. 2008).

Second aim of our investigation was to see whether specific dimensions of depressive disorder show significant correlation with serum BDNF levels. Even though our results don't suggest that for reasons ascribed above, our neurobiological starting point was the fact some of the phenotypes in depressed patients, including the melancholic phenotype, related to even more significant disorder on the hypothalamic-pituitary-adrenal (HPA) axis when compared with some other depressive manifestations (Pintor et al. 2009). In similar fashion, Dell'Osso et al. (2010) showed negative correlation between the HAMD-17 item psychomotor retardation and BDNF concentration which is an interesting result considering the fact psychomotor retardation represents one of the most significant depression symptoms and is generally related to the severity of illness (Benazzi et al. 2002). Moreover, some studies haven't found a connection between serum BDNF levels and broader symptoms and clinical features of depression like suicide ideation and the presence of comorbid anxiety (Molendijk et al. 2011). Further research is needed in order to shed some new light on the role of BDNF in different dimensions and clinical features of unipolar depression.

Recommendations concerning future investigations could be named. Studies, including this one, that showed no correlation between BDNF levels and severity of depression used serum instead of plasma BDNF so it would be of interest to further explore this

issue. Also, one might expect slightly different results by using some other psychological self-report measure of depression, especially because some recent validation studies have shown unstable psychometric properties of HAMD-17 leading to vague meaning of its total score (Bagby et al. 2004). Because some studies have shown gender specific findings in this area, focus on gender as a potential factor should be considered in order to resolve these inconsistencies.

CONCLUSION

Based on the results of this study, it is concluded that serum BDNF levels are not related to the severity of depression and its specific symptomatic dimensions. These findings support the idea of a complex relationship between BDNF concentration at the periphery and in the CNS.

REFERENCES

1. Aydemir C, Yalcin ES, Aksaray S, Kisa C, Yildirim SG, Uzbay T, et al.: Brain-derived neurotrophic factor (BDNF) changes in the serum of depressed women. *Prag Neuropsychopharmacol Biol Psychiatry* 2006; 30:1256-60.
2. Aydemir O, Devenci A, Taskin OE, Taneli F, Esen-Danaci A: Serum brain-derived neurotrophic factor level in dysthymia: a comparative study with major depressive disorder. *Prog Neuropsychopharmacol Biol Psychiatry* 2007; 31:1023-6.
3. Bagby RM, Ryder AG, Schuller DR, Marshall MB: The Hamilton Depression Rating Scale: Has the Gold Standard Become a Lead Weight? *Am J Psychiatry* 2004; 161:2163-2177.
4. Barde YA, Edgar D, Thoenen H: Purification of a new neurotrophic factor from mammalian brain. *EMBO J*. 1982; 1:549-53.
5. Benazzi F: Psychomotor changes in melancholic and atypical depression: 1976 unipolar bipolar-II II subtypes. *Psychiatry Res*. 2002; 112:211-20.
6. Brunoni AR, Lopes M, Fregni F: A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. *Int J Neuropsychopharmacol*. 2008; 11:1169-80.
7. Bus BAA, Molendijk ML, Penninx BJWH, Buitelaar JK, Kenis G, Prickaerts J, et al.: Determinants of serum brain-derived neurotrophic factor. *Psychoneuroendocrinology* 2011; 36:228-39.
8. Ciammola A, Sassone J, Cannella M, Calza S, Poletti B, Frati L, et al.: Low brain-derived neurotrophic factor (BDNF) levels in serum of Huntington's disease patients. *Am J Med Genet B Neuropsychiatr Genet*. 2007; 144:574-7.
9. Cole JC, Motivala SJ, Dang J, Lucko A, Lang N, Levin MJ, Oxman MN, Irwin MR: Structural Validation of the Hamilton Depression Rating Scale. *J Psychopath Behav Assess*. 2004; 26:241-254.
10. Dell'Osso L, Del Debbio A, Veltri A, Bianchi C, Roncaglia I, Carlini M, et al.: Association between Brain-Derived Neurotrophic Factor Plasma Levels and Severity of the

- illness, Recurrence and Symptoms in Depressed Patients. *Neuropsychobiology* 2010; 62:207-12.
11. Gervasoni N, Aubry JM, Bondolfi G, Osiek C, Schwald M, Bertschy G, et al.: Partial normalization of serum brain-derived neurotrophic factor in remitted patients after a major depressive episode. *Neuropsychobiology* 2005; 51:234-8.
 12. Gonul AS, Akedeniz F, Taneli F, Donat O, Eker C, Vahip S: Effect of treatment on serum brain-derived neurotrophic factor levels in depressed patients. *Eur Arch Psychiatry Clin Neurosci*. 2005; 255:381-6.
 13. Grassi-Oliveira R, Stein LM, Lopes RP, Teixeira AL, Bauer ME: Low plasma Brain-derived neurotrophic factor and childhood physical neglect are associated with verbal memory impairment in major depression – a preliminary report. *Biol Psychiatry* 2008; 64:281-285.
 14. Gustafsson G, Lira CM, Johansson J, Wisen A, Wohlfart B, Ekman R, Westrin A: The acute response of plasma brain-derived neurotrophic factor as a result of exercise in major depressive disorder. *Psychiatry Res*. 2009; 169:244-8.
 15. Hallbook F, Ibanez CF, Persson H: Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in *Xenopus* ovary. *Neuron* 1991; 6:845–58.
 16. Hamilton M: A rating scale for depression. *J Neurol Neurosurg Psychiatry* 1960; 23:56-62.
 17. Hashimoto K, Iwata Y, Nakamura K, Tsujii M, Tsuchiya KJ, Sekine Y, et al.: Reduced serum levels of brain-derived neurotrophic factor in adult male patients with autism. *Prog Neuropsychopharmacol Biol Psychiatry* 2006; 30:1529-31.
 18. Huang TL, Lee CT, Liu YI: Serum brain-derived neurotrophic factor levels in patients with major depression: effect of antidepressants. *J Psychiatric Res*. 2007; 45:521-5.
 19. Huang EJ, Reichardt LF: Neurotrophins: roles in neuronal development and function. *Ann Rev Neurosci*. 2001; 24:677–736.
 20. Ikeda Y, Yahata N, Ito I, Nagano M, Toyota T, Yoshikawa T, et al.: Low serum levels of brain-derived neurotrophic factor and epidermal growth factor in patients with chronic schizophrenia. *Schizophr Res*. 2008; 101:58-66.
 21. Jakovljević M: Agomelatine as chronopsychopharmaceutics restoring circadian rhythms and enhancing resilience to stress: a wishful thinking or an innovative strategy for superior management of depression? *Psychiatria Danubina* 2011; 23:2-9.
 22. Jevtović S, Šagud M, Mihaljević-Peješ A: Hamiltonova ocjenska ljestvica za depresiju. U: Antidepresivi u kliničkoj praksi. Ur. A.Mihaljević-Peješ & M.Šagud. Medicinska naklada, Zagreb 2011; 63-66.
 23. Karege F, Bondolfi G, Gervasoni N, Schwald M, Aubry JM, Bertschy G: Low brain-derived neurotrophic factor (BDNF) levels in serum of depressed patients probably result from lowered platelet BDNF release unrelated to platelet reactivity. *Brain Res Mol Brain Res*. 2005; 136:29-37.
 24. Karege F, Bondolfi G, Gervasoni N, Schwald M, Aubry JM, Bertschy G: Low brain-derived neurotrophic factor (BDNF) levels in serum of depressed patients probably result from lowered platelet BDNF release unrelated to platelet reactivity. *Biol Psychiatry* 2005; 57:1068–1072.
 25. Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry JM: Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psychiatry Res*. 2002; 109:143-8.
 26. Kauer-Sant'Anna M, Tramontina J, Andreazza AC, Cereser K, da Costa S, Santini A et al.: Traumatic life events in bipolar disorder: impact on BDNF levels and psychopathology. *Bipolar Disord*. 2007; 9: 128-135.
 27. Klein AB, Williamson R, Santini MA, Clemmensen C, Ettrup A, Rios M et al.: Blood BDNF concentrations reflect brain-tissue BDNF levels across species. *Int J Neuropsychopharmacol*. 2011; 14:347-353.
 28. Lee BH, Kim H, Park SH, Kim Yk: Decreased plasma BDNF level in depressive patients *J Affect Disord*. 2007; 101:239–44.
 29. Lu B, Je HS: Neurotrophic regulation of the development and function of the neuromuscular synapses. *J Neurocytol*. 2003; 32:931–41.
 30. Lu B, Woo N: Trophic factors in synaptic plasticity and memory. *Neuroscientist*. 2006; 12:43–56.
 31. Lommatzsch K, Zingler C, Schuff-Werner P: The impact of age, weight and gender on BDNF levels in human platelets and plasma. *Neurobiol Aging* 2005; 26:115–123.
 32. Machado-Vieira R, Dietrich MO, Leke R, Cereser VH, Zanatto V, Kapczinski F, et al.: Decreased plasma brain derived neurotrophic factor levels in unmedicated bipolar patients during manic episode. *Biol Psychiatry* 2007; 61:142-4.
 33. Maisonpierre PC, Belluscio L, Squinto S, Ip NY, Furth ME, Lindsay ME, et al.: Neurotrophin-3: a neurotrophic factor related to NGF and BDNF. *Science* 1990; 247:1446–51.
 34. McAllister AK, Katz LC, Lo DC: Neurotrophins and synaptic plasticity. *Annu Rev Neurosci*. 1999; 22:295–318.
 35. Mitoma M, Yoshimura R, Sugita A, Umene W, Hori H, Nakano H, Ueda N, Nakamura J: Stress at work alters serum brain-derived neurotrophic factor (BDNF) levels and plasma 3-methoxy-4-hydroxyphenylglycol (MHPG) levels in healthy volunteers: BDNF and MHPG as possible biological markers of mental stress? *Prog Neuropsychopharmacol Biol Psychiatry* 2008; 32:679-85.
 36. Molendijk ML, Bus BAA, Spinhoven P, Penninx BWJH, Kenis G, Prickaerts J, Oude Voshaar RC, Elzinga BM: Serum levels of brain-derived neurotrophic factor in major depressive disorder: state-trait issues, clinical features and pharmacological treatment. *Mol Psych*. 2011; 16:1088-1095.
 37. Monteleone P, Serritella C, Martiadis V, Maj M: Decreased levels of serum brain-derived neurotrophic factor in both depressed and euthymic patients with unipolar depression and in euthymic patients with bipolar I and II disorders. *Bipolar Disord*. 2008; 10:95-100.
 38. Nakazato M, Hashimoto K, Shimizu E, Kumakiri C, Koizumi H, Okamura N, et al.: Decreased levels of serum brain-derived neurotrophic factor in female patients with eating disorders. *Biol Psychiatry* 2003; 54:485-90.
 39. Palomino A, Vallejo-Illarramendi A, González-Pinto A, Aldam A, González Gómez C, Mosquera F, et al.: Decreased levels of plasma BDNF in first episode schizophrenia and bipolar disorder patients. *Schizophr Res*. 2006; 86:321–322.

40. Pang PT, Teng HK, Zaitsev E, Woo N, Sakata K, Zhen S et al.: Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. *Science* 2004; 306:487-91.
41. Piccinni A, Marazziti D, Catena M, Domenici L, Del Debbio A, Bianchi C, et al.: Plasma and serum brain-derived neurotrophic factor (BDNF) in depressed patients during 1 year of antidepressant treatments. *J Affect Disord*. 2008; 105:279-83.
42. Pintor L, Torres X, Navarro V, Martinez de Osaba MA, Matrai S, Gasto C: Prediction of relapse in melancholic depressive patients in a 2-year follow-up study with corticotropin releasing factor test. *Prog Neuropsychopharmacol Biol Psychiatry* 2009; 33:463-469.
43. Poo MM: Neurotrophins as synaptic modulators. *Nat Rev Neurosci*. 2001; 2:24-32.
44. Sen S, Duman R, Sanacora G: Seruma brain-derived neurotrophic factor, depression, and antidepressant medications: Meta-analyses and implications. *Biol Psychiatry* 2008; 64:527-32.
45. Sheehan DV, Lecrubier Y, Sheehan KH et al.: The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* 1998; 59:22-33.
46. Shimizu E, Hashimoto K, Okamura N, Koike K, Komatsu N, Kumakiri C, et al.: Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. *Biol Psychiatry* 2003; 54:70-5.
47. Shvartzman P, Weiner Z, Vardy D, Friger M, Sherf M, Biderman A: Health services utilization by depressive patients identified by the MINI questionnaire in a primary care setting. *Scand J Prim Health Care* 2005; 23:18-25.
48. Sozeri-Varma G, Enli Y, Toker-Ugurlu T, Alacam H, Kalkan-Oguzhanoglu N: Decreased serum BDNF levels in major depressive patients. *Neurology, Psychiatry and Brain Research*. 2011; (In Press).
49. Tyler WJ, Alonso M, Bramham CR, Pozzo-Miller LD: From acquisition to consolidation: on the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. *Learn Mem*. 2002; 9:224-37.
50. Yasutake C, Kuroda K, Yanagawa T, Okamura T, Yoneda H: Serum BDNF, TNF-alpha and IL-1beta levels in dementia patients: Comparison between Alzheimer's disease and vascular dementia. *Eur Arch Psychiatry Clin Neurosci*. 2006; 256:402-6.
51. Yoshimura R, Mitoma M, Sugita A, Hori H, Okamoto T, Umene W, et al.: Effects of paroxetine or milnacipran on serum brain-derived neurotrophic factor in depressed patients. *Prog Neuropsychopharmacol Biol Psychiatry* 2007; 31:1034-7.

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