

DIFFERENT SERUM BDNF LEVELS IN DEPRESSION: RESULTS FROM BDNF STUDIES IN FYR MACEDONIA AND BULGARIA

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SUMMARY

Background: A growing body of evidence shows that brain-derived neurotrophic factor (BDNF) plays a role in depressive disorder. Serum BDNF levels are lower in depressed patients and they increase after a long course of antidepressant treatment. Our study aims to test the effect of antidepressant treatment on serum BDNF levels in patients with a depressive episode, after they have achieved remission in two studies in Macedonia and Bulgaria.

Subjects and methods: In the Macedonian study 23 patients were included (11 female, 12 male) diagnosed with a first depressive episode according to ICD-10, as well as 23 control subjects age- and sex-matched without a history of psychiatric disorder. In the Bulgarian study 10 female patients with depression and 10 control subjects were included. We have applied the Hamilton Depression Rating Scale (HDRS) to assess depression severity. Blood samples were collected before antidepressive treatment and after remission was achieved (decrease to 7 points or less on HDRS).

Results: In the Macedonian study, mean serum BDNF level at baseline was 13.15 ± 6.75 ng/ml and the mean HDRS score was 28.52 ± 4.02 . Untreated depressed patients showed significantly lower serum BDNF levels compared to the control group (25.95 ± 9.17 ng/ml). After remission was achieved, the mean serum BDNF level was 24.73 ± 11.80 ng/ml whereas the mean HDRS score was 7.04 ± 3.15 . After 8 weeks of treatment there was no statistically significant difference in the serum BDNF levels between the two groups. In the Bulgarian study, baseline mean serum BDNF levels were 26.84 ± 8.66 ng/ml, after 3 weeks treatment and remission was achieved mean serum BDNF levels were 30.33 ± 9.25 ng/ml and in the control group mean serum BDNF levels were 25.04 ± 2.88 ng/ml. Integrated results showed baseline mean serum BDNF levels of 17.30 ± 9.66 ng/ml, after achieved remission 26.43 ± 11.25 ng/ml and in the control group mean serum BDNF levels of 25.68 ± 7.76 ng/ml.

Conclusion: The Bulgarian results showed no statistical difference between the depressed group and controls. The Integrated results and the Macedonian study supported previous findings of low BDNF levels in untreated depressive patients compared to healthy controls, and that those levels increase after antidepressant treatment. These results may suggest that low serum levels of BDNF are a state abnormality that is evident during depression and normalizes during remission.

Key words: depressive disorder – BDNF - state trait biological marker - FYR Macedonia - Bulgaria

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INTRODUCTION

Depressive disorder is a serious illness that affects 17% of population in some life period, and therefore places a heavy social and economic burden over society (Kessler et al. 1994, Belmaker & Agam 2008). Lifetime prevalence of depressive disorder is estimated between 6% and 16%. The etiology of depressive disorder is not completely understood, but there is evidence in support of complex interactions of biological, genetic, psychosocial and environmental factors. The depression risk is associated with a combination of many etiological factors (Costello et al. 2002, Merikangas et al. 2002, Nestler et al. 2002). As well as the etiology, the neurobiology that underlies mood disorders is currently not precisely determined.

Brain-derived neurotrophic factor (BDNF) involvement in depressive disorder has been a focus of intensive research for the last decade. BDNF belongs to a neurotrophic family of growth factors and affects neuronal survival and function. BDNF is detectable in

blood, although its concentration in brain tissue is higher (Yamamoto & Gurney 1990, Radka et al. 1996).

It has been reported that BDNF may pass the blood-brain barrier (Pan et al. 1998) and that BDNF serum and brain levels go through similar changes in maturation and ageing in rats, (Karege et al. 2002) which indicates that serum BDNF levels reflect brain BDNF levels.

Karege et al. (2005) were the first to report significantly reduced serum BDNF levels in untreated patients with major depressive disorder, and that serum BDNF levels are in negative correlation with depression severity. Shimizu et al. (2003) reported that BDNF levels in patients with major depressive disorder who were not treated with antidepressants, are significantly lower than those in patients who were treated and healthy controls; they have also shown that serum levels of BDNF are in negative correlation with severity of depression.

Sen et al. (2008) meta-analysis provides strong evidence that BDNF levels are lower in depressive patients compared to healthy controls ($p < 6.8 \times 10^{-8}$), and that BDNF levels are significantly increased

($p=0.003$) after antidepressive treatment. Brunoni et al. (2008) meta-analysis, similarly showed that BDNF levels are significantly increased after antidepressive treatment (effect size: 0.62), and that significant correlation exists between ($p=0.02$) BDNF changes and changes in depression rating scale results. However, findings about the association between BDNF levels and severity of depression are not ubiquitous (Jevtovic et al. 2011). There are still some questions that remain unanswered, which makes the neurotrophin hypothesis at best incomplete (Groves 2007).

Our study aims to test the effect of antidepressant treatment on serum BDNF levels in patients with a depressive episode, after remission has been achieved in two separate studies in Macedonia and Bulgaria. We present the separate results from both studies as well as the integrated results in order to emphasize the existing difference in the three groups of samples.

SUBJECTS AND METHODS

Macedonian study

Twenty three patients (11 female, 12 male) diagnosed with a first depressive episode according to ICD-10 were included in this study. Average age of the experimental group was 44.22 (20-72) years. The severity of depression was assessed with the structured interview of the Hamilton Depression Rating Scale (HDRS). The control group consisted of 23 subjects age- and sex-matched without a history of psychiatric disorder (average age 44.04 years). Patients were treated with sertraline, paroxetine or venlafaxine. Blood samples were collected at the baseline and after patients achieved remission (decrease to 7 points or less on HDRS, average time between two measurements 8 weeks). Serum BDNF levels were measured using the BDNF Emax Immunoassay System kit (Promega; Madison, WI, USA) according to the manufacturer's instructions. The study was approved by the Ethics Committee of the Medical Faculty, University "Sts. Cyril and Methodius" Skopje, and written informed consent was obtained from all participants.

Bulgarian study

In the Bulgarian study 10 female patients with depression and 10 control subjects were included. The average age of the depressed group was 51.2 years (38-60) and of the control group was 38 years (32-42). Blood samples were collected at the baseline and after patients achieved remission (average time between two measurements 3 weeks). Serum BDNF levels were measured using the Quantikine Human BDNF Immunoassay (R&D Systems) according to the manufacturer's instructions.

Both studies followed the same procedure according to the manufacturers' instructions, in short, 96-well micro plates were coated with anti-BDNF monoclonal

antibody and incubated at 4°C for 18 h. The plates were incubated in a blocking buffer for 1 h at room temperature. The samples diluted with assay buffer 100-times and BDNF standards were kept at room temperature under conditions of horizontal shaking for 2 h, followed by washing with washing buffer. The plates were incubated with antihuman BDNF polyclonal antibody at room temperature for 2 h and washed with the washing buffer. Then the plates were incubated with anti-IgY antibody conjugated to horseradish peroxidase for 1 h at room temperature, and incubated in peroxidase substrate and tetramethylbenzidine solution to induce a colour reaction. The reaction was stopped with 1 mol/L hydrochloric acid. The absorbance at 450 nm was measured with automated micro plate reader. Measurements were performed in duplicate. The standard curve was linear from 5 pg/mL to 5000 pg/mL. Cross-reactivity to related neurotrophins (NT-3, NT-4, NGF) was less than 3%. Intra- and inter-assay coefficients of variation were 5% and 7%, respectively. The recovery rate of the exogenous added BDNF in the measured plasma samples was more than 95%.

Statistical analysis

The data were analyzed with SPSS 17.0 statistical program and are presented as mean value and SD. Student's t-test was used for the continuous variables. Pearson's correlation coefficient has been used to examine relationships between pairs of variables, p values <0.05 were considered significant. We calculated the different variables with an analysis of variance (ANOVA).

RESULTS

The results of serum BDNF levels and HDRS score in the samples of both studies are shown in table 1. Group serum BDNF levels in Macedonian samples are shown in figure 1. In the Macedonian sample ANOVA shows a statistically significant difference between the two samples (relapse/remission) ($F(2,66)=12.806$, $p<0.017$). No significant correlations emerged between serum BDNF levels and age of onset of the illness, duration of the illness and number of depressive episodes. 14 patients were treated with sertraline, 6 with paroxetine and 3 with venlafaxine. There was no significant difference between BDNF levels between the 3 groups treated with different antidepressive medication ($p>0.5$).

In the Bulgarian sample ANOVA showed no statistically significant difference between the two samples (relapse/remission) ($F(2,27)=1.286$, $p=0.293$). Group serum BDNF levels are shown in figure 2.

In the integrated results of both studies ANOVA showed statistically significant difference between the two samples (relapse/remission) ($F(2,64)=12.985$, $p<0.001$). Group serum BDNF levels are shown in figure 3.

Table 1. Results of serum BDNF levels and HDRS score in both groups

		BDNF Pre-treatment ng/ml	BDNF Post-treatment ng/ml	BDNF Controls ng/ml	HDRS 1	HDRS 2
Macedonian results	Mean ± SD	13.15± 6.75	24.73±11.80	25.95±9.17	28.52±4.02	7.04±3.15
Bulgarian results	Mean ± SD	26.84±8.66	30.33±9.25	25.04±2.88		
Integrated results	Mean ± SD	17.30±9.66	26.43±11.25	25.68±7.76		

BDNF Pre-treatment – BDNF serum levels in depressed patients before treatment
 BDNF Post-treatment – BDNF serum levels in depressed patients after treatment
 BDNF Controls – BDNF serum levels in control group
 HDRS 1 – Hamilton Depression Rating Scale results in depressed patients before treatment
 HDRS 2 – Hamilton Depression Rating Scale results in depressed patients after treatment

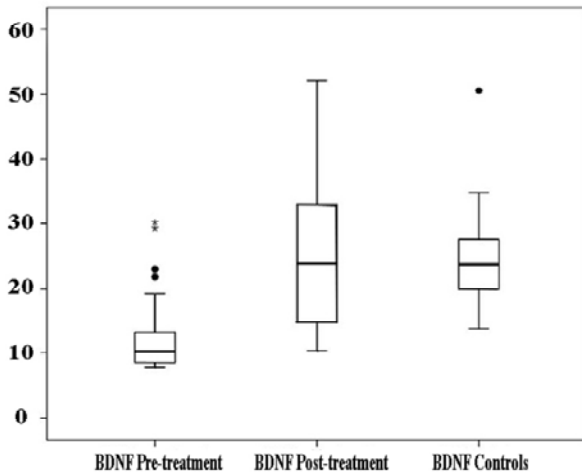


Figure 1. Macedonian sample - BDNF levels (ng/ml): pre-treatment, post-treatment and controls

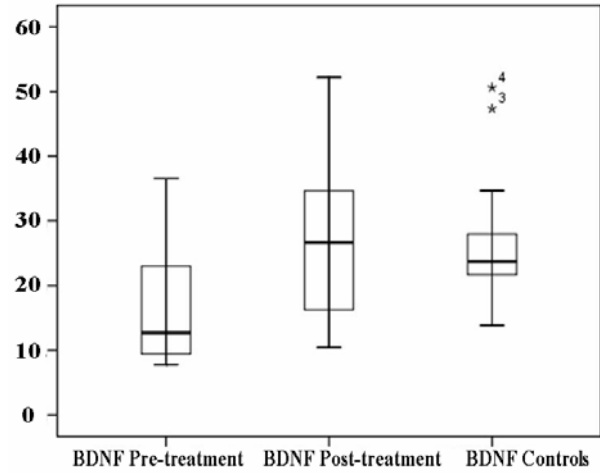


Figure 3. Integrated Result - BDNF levels (ng/ml): pre-treatment, post-treatment and controls

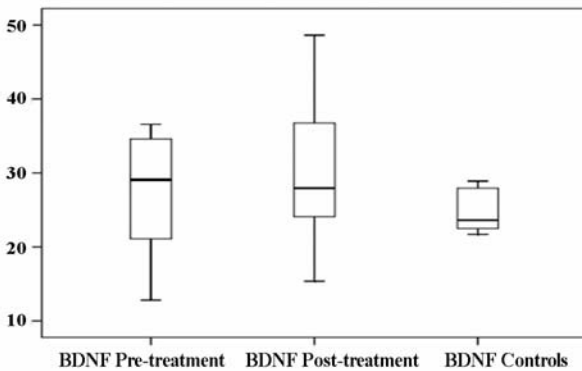


Figure 2. Bulgarian sample - BDNF levels (ng/ml): pre-treatment, post-treatment and controls

DISCUSSION

Both studies used similar methodology with a common goal to examine the effect of the depressive episode on the serum BDNF levels as well as the effect of chronic antidepressive treatment.

In the Bulgarian sample we found no statistically significant difference between serum BDNF levels of depressed patients before and after treatment. An ANOVA shows no statistically significant difference between the 3 samples. There are several possible explanations for these results. The sample is too small to

draw definite conclusions, but it is surprising that the mean BDNF level in depressed patients is almost the same as of healthy controls. Another reason for the statistically not significant BDNF results in depressed patients after treatment may be the short duration of the treatment course (3 weeks). The puzzling results concerning this sample of patients whose BDNF levels did not increase after treatment requires further exploration. For instance, it is noteworthy that HPA axis dysregulation has been observed to diminish during antidepressant treatment among remitted patients (Zobel et al. 2001).

In the Macedonian sample we have found a statistically significant difference ($p=0.000091$) between serum BDNF levels in depressed patients at baseline, compared to BDNF levels after treatment, and compared to healthy control subjects ($p<0.05$). Furthermore, antidepressant treatment increased BDNF levels in depressed patients (24.73 ± 11.80) close to the levels of healthy controls (25.95 ± 9.17) and there is no statistically significant difference between serum BDNF levels ($p=0.349$).

However, integrated results of both studies with altogether 33 patients, show lower levels of BDNF while depressive symptoms are evident (mean 17.30 ± 9.66 ng/ml). BDNF serum levels increased significantly ($p<0.001$) in patients with improved depressive symptoms after antidepressive treatment (mean 26.43 ± 11.25).

The integrated results show that chronic antidepressive treatment can significantly increase low serum BDNF levels in patients with depressive disorder when patients achieve remission. When compared with the control group, serum BDNF level is lower in untreated depressive patients, and antidepressive treatment increases serum BDNF level in depressive patients to the level of healthy controls. These findings correspond to previously published studies on serum BDNF levels, after Karege et al. (2002) were the first to publish lower serum BDNF levels in depressive patients free of antidepressive treatment (Gervasoni et al. 2005, Gonul et al. 2005, Karege et al. 2005, Aydemir et al. 2006, Yoshimura et al. 2007, Huang et al. 2007, Piccinni et al. 2010, Kimpton 2012, Karlović et al. 2013, Ladea & Bran 2013). Our results may indicate that a low serum BDNF level may be an important feature of depression. The finding of lower BDNF levels in depressed patients before treatment compared to BDNF levels after treatment indicates that this neurotrophic factor has a pivotal role in depressive disorder. Our results suggest that low serum levels of BDNF are a state abnormality that is evident during depression and normalizes during remission, congruent to the results of Molendijk et al. (2011).

Some limitations must be considered when interpreting these results. Firstly, the sample size is relatively small. A second limitation of this study is that we measured circulating BDNF; so the question arises whether peripheral BDNF reflects neurotrophin levels in the brain (Monteleone et al. 2008). It has been demonstrated that, in the rat, brain and serum BDNF concentrations undergo parallel changes during maturation and aging (Karege et al. 2005); furthermore, BDNF has been shown to be able to cross the blood–brain barrier (Pan et al. 1998). All these data support the view that peripheral BDNF changes reflect similar changes in the brain, where its modulatory role on affective symptomatology is likely to be exerted. Although the source of circulating BDNF is still unknown, platelets have shown to be able to bind, store and release BDNF upon activation (Fujimura et al. 2002).

There was no statistically significant difference in BDNF levels between patients treated with sertraline, paroxetine or venlafaxine. These findings are in contradiction to the Hellweg et al. (2008) study who found that changes in BDNF serum concentrations as a result of antidepressant therapy depend on the antidepressant instead of being a general characteristic of response to antidepressant treatment.

All of our patients suffered from a first episode of depression that in average lasted 4 months before antidepressive treatment. Decreased levels of BDNF indicate that even first signs of depression may have an impact on neuronal activity. Antidepressive treatment appears to normalize BDNF levels, so it might be safe to assume that earlier pharmacological intervention is neuroprotective.

The present findings confirm previously independent studies reporting lowered circulating BDNF in patients with MDD. Further detailed studies are needed to ascertain whether such an alteration may represent a trait vulnerability marker for affective disorders.

CONCLUSION

We can conclude from the Bulgarian study that not all BDNF results are consistent with the previous findings and that we should try to test BDNF levels in depression in larger samples. The findings of the Macedonian study indicate that BDNF levels are significantly lower in untreated depressive patients compared to healthy controls, and that those levels increase after antidepressant treatment. These results may be interpreted as positive effects of antidepressant treatment on neuroplasticity and depressive symptoms. The integrated results of both studies indicate that BDNF serum levels increased significantly in patients with improved depressive symptoms and antidepressive treatment. The effects of different antidepressive medications are associated with BDNF increase, which may indicate that BDNF is a “common final pathway” for different antidepressive approaches.

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Conflict of interest : None to declare.

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