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Effect of vortioxetine vs. escitalopram on plasma BDNF and platelet serotonin in depressed patients

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Highlights

- vortioxetine increased plasma BDNF levels, while escitalopram had no effects
- escitalopram induced greater decrease in platelet 5-HT levels than vortioxetine
- response to vortioxetine was not predicted by baseline plasma BDNF/platelet 5-HT
- baseline platelet 5-HT concentration predicted escitalopram response
- both drugs significantly reduced depressive symptoms in depressed patients

Abstract

Escitalopram and vortioxetine are efficacious antidepressants. They directly target serotonin (5-HT) system, but vortioxetine mechanism of action is distinct from the one of selective serotonin reuptake inhibitors (SSRIs). Treatment with SSRIs decrease platelet 5-HT concentration and increase peripheral brain-derived neurotrophic factor (BDNF) levels. Since vortioxetine has a multimodal mechanism of action, it is expected to have a greater effect on circulatory BDNF concentration, compared to conventional antidepressants. This longitudinal study aimed to explore and compare the effects of 4-weeks of treatment with vortioxetine and escitalopram on plasma BDNF and platelet 5-HT concentration in patients with major depressive disorder (MDD). The results revealed that vortioxetine significantly increased plasma BDNF concentration ($p=0.018$) and significantly decreased platelet 5-HT concentration ($p<0.001$). Treatment with escitalopram significantly decreased platelet 5-HT concentration ($p<0.001$), but it did not affect plasma BDNF concentration ($p=0.379$). Response to vortioxetine was not predicted by baseline plasma BDNF or platelet 5-HT concentration, but response to escitalopram was predicted by baseline platelet 5-HT concentration. These effects might be due to vortioxetine unique mechanism of action, but the clinical implications are unclear. It remains to be determined whether this finding extends during long-term vortioxetine treatment, and which, if any, clinical effects emerge from BDNF increase.

Keywords

depression; escitalopram; plasma BDNF; platelet serotonin; patients; vortioxetine

Declaration of interest

The Authors declare that there is no conflict of interest.

Abbreviations

BDNF=brain derived neurotrophic factor; DSM-5=Diagnostic and Statistical Manual of Mental Disorders; ELISA=enzyme-linked immunosorbent assay; HAMD=Hamilton Rating Scale for Depression; MADRS= Montgomery-Åsberg Depression Rating Scale; MDD=major depressive disorder; 5-HT=serotonin; SERT=serotonin transporter; SSRIs= selective serotonin reuptake inhibitors; WHO=World Health Organization

1 Introduction

Depression is a common, and often severe and disabling disorder. It is ranked by WHO as the single largest contributor to global disability (WHO, 2017). Antidepressants are the cornerstone of treatment for moderate to severe depression, and all drugs from this class are more effective than placebo (Cipriani et al., 2018). Despite extensive research, their mechanism of action remains incompletely understood. While antidepressants initially modulate the activity of monoaminergic systems, clinical response is related to more complex downstream mechanisms, which involve, among others, amplification of neurotrophic signaling in the hippocampus (Björkholm and Monteggia, 2016). Among neurotrophins, brain-derived neurotrophic factor (BDNF) is related to neuronal survival, synaptic signaling and synaptic consolidation, and to a treatment response in depression (Jiang et al., 2017).

Different experimental stressors decrease hippocampal BDNF concentrations (Burstein et al., 2017; Lu et al., 2018; Park et al., 2018; Aguiar et al., 2019; Ozbeyli et al., 2019; Seo et al., 2019), and antidepressants, such as escitalopram, have been reported to either normalize (Burstein et al., 2017; Seo et al., 2019) BDNF function or prevent (Park et al., 2018) the reduction of BDNF concentrations. Although first detected in the brain, BDNF also exists at the periphery, being mainly stored in platelets. Because central BDNF concentrations are difficult to assess in humans, there is a great interest in peripheral BDNF measures in relation to psychiatric disorders, including depression. Since the first study showing the low serum BDNF concentration in patients with major depressive disorder (MDD) compared to controls has been reported (Karege et al., 2002), accumulating evidence suggests that alterations in peripheral BDNF concentration are associated with both depression pathophysiology and response to antidepressants (Jiang et al., 2017). Circulatory BDNF concentration could be measured in plasma, serum, platelets and the whole blood. Unlike in serum, BDNF concentration in plasma represents free and biologically active form or state-dependent marker (Serra-Millàs, 2016; Polyakova et al., 2017). Plasma BDNF concentration may change during specific interventions in patients with MDD (Sagud et al., 2016) and in healthy respondents (Cho et al., 2012), but appear stable over time in healthy individuals undergoing no intervention (Gejl et al., 2019; Sustar et al., 2019). It has been claimed that antidepressants should be given at least four 4 weeks to restore the decreased BDNF function (Lee and Kim, 2010).

While selective serotonin reuptake inhibitors (SSRIs), like escitalopram, continue to be the most frequently prescribed antidepressants world-wide, vortioxetine was recently introduced as a new option for the treatment of MDD. Though both escitalopram and vortioxetine directly target serotonergic system, vortioxetine's mechanism of action appears to be distinct from the SSRIs. All SSRIs, due to the potent serotonin transporter (SERT) blockade, induce alterations of brain serotonin (5-hydroxytryptamine, 5-HT) extracellular levels and transmission (Fritze et al., 2017), and diminish platelet 5-HT content (Muck-Seler et al., 2002; Pivac et al., 2003; Knorr et al., 2019). Therefore, platelet 5-HT represents an easy obtainable peripheral marker of the treatment response to SSRIs, such as escitalopram. Unlike SSRIs, vortioxetine exerts its effects only partially by the SERT blockade. While SERT inhibition by vortioxetine still appeared sufficient to significantly decrease platelet 5-HT concentration (Sagud et al., 2016), it is unknown if the extent of such reduction is similar to those of SSRIs, or weaker. The other difference between vortioxetine and escitalopram might arise from the additional vortioxetine's mechanism of action, such as the 5HT1A receptor partial agonism. This feature might result in distinct effects on BDNF, that were reported in the animal models. For example, while both the preferential 5-HT1A postsynaptic receptor agonist and escitalopram restored BDNF after ischemic injury in mice hippocampus, only 5-HT1A postsynaptic receptor agonist NLX-101 (a.k.a. F15599) increased BDNF protein levels in the prefrontal cortex (Aguiar et al., 2019).

Based on these findings, vortioxetine might be also expected to have stronger effects on circulatory BDNF concentration compared to conventional antidepressants.

So far, only two clinical studies addressed vortioxetine effects on peripheral BDNF concentration. First trial reported increase in serum BDNF levels after one-year of vortioxetine treatment, with more pronounced BDNF elevation in patients who received cognitive-behavioral treatment in addition to vortioxetine, compared to those who received vortioxetine alone (Yan et al., 2019). In our pilot, uncontrolled trial, 4-week vortioxetine treatment significantly increased plasma BDNF concentration in patients with MDD compared to BDNF values in healthy control subjects (Sagud et al., 2016). However, it is unknown if the magnitude of increase in peripheral BDNF concentration in patients with depression differs in those treated with vortioxetine or SSRIs.

Both escitalopram and vortioxetine are amongst the most efficacious antidepressants. The most recent meta-analysis has shown that agomelatine, amitriptyline, escitalopram, mirtazapine, paroxetine, venlafaxine, and vortioxetine were more effective, whereas agomelatine, citalopram, escitalopram, fluoxetine, sertraline, and vortioxetine were more tolerable than other antidepressants, respectively (Cipriani et al., 2018).

Given the unique vortioxetine mechanism of action, we hypothesized that vortioxetine would induce greater elevation of plasma BDNF concentration than escitalopram, and lower reduction of platelet 5-HT content, in patients with MDD.

2 Methods

2.1 Patient population and study design

Patients included in the study were diagnosed with MDD, aged 18 years or older, who were either drug-naïve, or free from antidepressants at least one month (or minimum of 6 weeks in the case of fluoxetine), having a minimal Hamilton Depression Rating Scale (HAM-D)-17 (Hamilton, 1960) score of 15. The diagnosis of MDD was confirmed using a structured clinical interview based on the DSM-5 criteria (APA, 2013). Patients with MDD were recruited at the Department of Psychiatry and Psychological Medicine, University Hospital Center Zagreb and Clinical Hospital Vrapce. All participants were Caucasians, and have signed the informed consent document, approved by the Ethics Committees of both institutions.

Exclusion criteria covered the presence of psychotic features, acute respiratory tract infections, treatment with tryptophan, St John's Wort, mood stabilizers, antipsychotics, hormonal replacement therapy or opioid analgesics one month prior to inclusion, benzodiazepine intake on daily basis, known treatment resistance to previous antidepressants, including escitalopram and vortioxetine, any comorbid psychotic disorder, bipolar affective disorder, alcohol or other substance dependence in previous 3 months, obsessive-compulsive disorder, eating disorder, dementia and other neurodegenerative disorders, severe somatic illness including poorly controlled arterial hypertension, diabetes, and thyroid disease, currently undergoing psychotherapy, except psychotherapeutic support that is usually a part of psychiatric examination and was similar in both patient groups.

Participants completed the Montgomery-Åsberg Depression Rating Scale (MADRS) (Montgomery and Åsberg, 1979) and HAM-D-17. Clinical response was defined as $\geq 50\%$ reduction from the baseline HAM-D scores (Kelley et al., 2018), after 4 weeks of treatment.

After baseline assessment, patients were randomized at 1:1 ratio, to vortioxetine or escitalopram. Starting doses were 5 or 10 mg once daily for both drugs. At the discretion of the psychiatrist-investigator, antidepressants might have been decreased to 5 mg daily, or increased up to 20 mg daily. However, the vast majority of patients received 10 mg of both drugs during

the entire study period. Regarding concomitant psychotropic agents, only low-dose benzodiazepines (up to 10 mg diazepam equivalent on “as needed” basis) were allowed. Study procedures are presented in Table 1.

2.2 Blood sampling

Blood samples were collected in 8.5 ml yellow-top Vacutainer tubes with 1.5 ml of acid citrate dextrose anticoagulant. Sampling was performed after the overnight fast, at 8 a.m., during routine laboratory visits. Platelets and platelet-poor plasma were obtained from whole blood samples by a series of centrifugation. Aliquots of platelet-poor plasma were stored at -20°C for BDNF concentration analysis. To decrease possible variability, all samples were processed within 1 h of being collected.

2.3 Platelet 5-HT concentration

Platelets were obtained by centrifugation from platelet-rich-plasma. To release their content, they were then disrupted by sonication. Platelet 5-HT concentrations were determined by ortho-phthalaldehyde-enhanced fluorometry. Briefly, blank samples, standards (5-HT) and platelet sonicates were incubated for 5 min with 10% ZnSO_4 and 1N NaOH at room temperature in order to precipitate proteins. After centrifugation, the supernatant was transferred into a new glass tube. After the addition of 1% L-cysteine and 0.01% ortho-phthalaldehyde, all samples were boiled for 10 min. The reaction was stopped using 1N NaOH. The fluorescence was measured on a Varian Spectrophotofluorimeter Cary Eclipse, with the excitation wavelength of 345 nm and emission wavelength of 485 nm. All samples were measured in duplicates. To correct the measured 5-HT concentration according to different platelet number in individual samples, platelet protein levels were measured by the method of Lowry et al. (1951).

2.4 Plasma BDNF concentration

BDNF concentration in platelet-poor plasma was determined using a commercial enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s instructions (Quantikine ELISA, R&D Systems, Minneapolis, USA). All plasma samples were diluted (1:2) using the diluent provided by the manufacturer and added, with standards and blanks, to the 96-well plate pre-coated with the monoclonal antibody specific for human BDNF. The plate was incubated for 2h at room temperature (RT) and, afterwards, a solution containing monoclonal antibodies conjugated to horseradish peroxidase was added to each well. After incubation (1h) at RT, the plate was washed with washing buffer to remove any unbound antibodies and a substrate solution was added. After 30 min of incubation in the dark at RT, the reaction was terminated using 2N sulfuric acid. A microplate reader was used to measure the absorbance of each sample, using a wavelength of 450 nm and corrected by absorbance at 570 nm. The intra- and inter-assay coefficients of variations were less than 10 %. The concentrations of each samples were calculated based on a standard curve. All samples were determined in duplicates.

2.5 Data analysis

Data was evaluated with Sigma Stat 3.5 (Jandel Scientific Corp., San Jose, California, USA). For all analyses, the significance level α was set to 0.025, since we tested two parameters in each treatment group (platelet 5-HT and plasma BDNF concentration). All tests used were two-tailed. The Kolmogorov-Smirnov test was used to determine the normality of the distribution of individual parameters analysed, including demographic and clinical parameters, platelet 5-HT and plasma BDNF concentration. Given the deviation of most of the studied parameters from the normal distribution, the clinical and demographic parameters, and the concentration of platelet 5-HT and plasma BDNF are shown as median and interquartile ranges between the 25th and 75th percentiles. Appropriate non-parametric statistical tests were used to compare the values of the various parameters studied. The Mann-Whitney U test was used to compare the

two independent data groups, and the Wilcoxon signed-rank test was used to compare the two dependent data sets. Analysis of covariance (ANCOVA) was used to test the differences between platelet 5-HT concentration and plasma BDNF levels after 4 weeks of therapy with two different antidepressants, in which the baseline value of platelet 5-HT concentration or plasma BDNF level was used as a covariate for correction. Prior to ANCOVA, log₁₀ data transformation was done due to data deviation from normal distribution.

G*Power 3 Software was used to determine a priori sample size and actual power. For the Wilcoxon signed-rank test (with $\alpha = 0.025$; power $(1 - \beta) = 0.95$; a large effect size $\omega = 0.80$), total desired sample size was 28, and the actual sample size was at least 60 per each group. For the Mann-Whitney U test (with $\alpha = 0.025$; power $(1 - \beta) = 0.95$; a large effect size $\omega = 0.80$), total desired sample size was 51 per group, and the actual sample size was 60 in escitalopram group and 61 for the group treated with vortioxetine. For ANCOVA (with $\alpha = 0.025$; power $(1 - \beta) = 0.95$; a medium effect size $\omega = 0.50$; number of groups = 2; number of covariates = 1), total desired sample size was 75, and the actual sample size was 121. Therefore, we had the appropriate sample size and statistical power to detect significant differences in the studied groups.

3 Results

Demographic and clinical parameters of patients, are presented in Table 2. There were no differences in the proportion of smokers and non-smokers between the groups ($p=0.752$), in the number of suicide attempts ($p=0.395$), and age ($p=0.164$). However, group treated with escitalopram had a higher proportion of female subjects ($p=0.015$), more patients with a first depressive episode ($p=0.009$), and a higher frequency of individuals with documented family history of depression ($p=0.017$). There was also a significant difference between the group treated with vortioxetine and escitalopram regarding the duration of illness ($p=0.019$), number of depressive episodes ($p=0.017$), HAMD-17 ($p=0.022$) and MADRS ($p=0.006$) baseline scores (Table 2). These results indicate that patients treated with vortioxetine had more severe symptoms of MDD and longer duration of illness (Table 2).

To eliminate the possible effects of age, gender and smoking on plasma BDNF concentration and platelet 5-HT concentration, before and after the treatment with vortioxetine or escitalopram, multiple linear regression analysis with age, gender, and smoking as independent variables was used. Multiple linear regression analysis with plasma BDNF concentration as dependent variable revealed a non-significant model at baseline ($F(3,57)=1.87$; $p=0.145$; $R_{adj}^2=0.042$), and after treatment with vortioxetine ($F(3,57)=2.89$; $p=0.043$; $R_{adj}^2=0.086$). These non-significant models were due to the lack of significant effects of age ($p=0.049$), gender ($p=0.451$), and smoking ($p=0.482$) on plasma BDNF concentration before treatment, and no significant effects of age ($p=0.272$), gender ($p=0.937$), and smoking ($p=0.042$) on plasma BDNF concentration after vortioxetine treatment. Multiple linear regression analysis with platelet 5-HT concentration as dependent variable showed a non-significant model at baseline ($F(3,57)=1.05$; $p=0.378$; $R_{adj}^2=0.002$), and after treatment with vortioxetine ($F(3,56)=1.71$; $p=0.175$; $R_{adj}^2=0.035$). These non-significant models were explained by the lack of significant effects of age ($p=0.163$), gender ($p=0.377$), and smoking ($p=0.631$) on platelet 5-HT concentration before treatment, and the lack of significant effects of age ($p=0.098$), gender ($p=0.771$), and smoking ($p=0.100$) on platelet 5-HT concentration after vortioxetine treatment.

Regarding the escitalopram group, multiple linear regression analysis with plasma BDNF concentration as dependent variable showed a non-significant model at baseline ($F(3,56)=0.91$; $p=0.441$; $R_{adj}^2=-0.004$), and after treatment with escitalopram ($F(3,56)=2.67$; $p=0.056$; $R_{adj}^2=0.078$). These non-significant models were the results of the lack of significant effects of age ($p=0.278$), gender ($p=0.172$), and smoking ($p=0.996$) on plasma BDNF concentration

before treatment, and the lack of significant effects of age ($p=0.138$), gender ($p=0.061$), and smoking ($p=0.132$) on plasma BDNF concentration after treatment with escitalopram. When platelet 5-HT concentration was used as dependent variable, there was again a non-significant model at baseline ($F(3,56)=1.04$; $p=0.382$; $R_{adj}^2=0.002$; multiple linear regression) and after treatment with escitalopram ($F(3,56)=0.65$; $p=0.584$; $R_{adj}^2=-0.018$). Models were not significant because of the non-significant effects of age ($p=0.163$), gender ($p=0.377$), and smoking ($p=0.631$) on platelet 5-HT concentration before treatment, and the lack of significant effects of age ($p=0.245$), gender ($p=0.919$), and smoking ($p=0.210$) on platelet 5-HT concentration after escitalopram treatment.

In patients with MDD, clinical parameters (HAMD-17 and MADRS scores), plasma BDNF and platelet 5-HT concentrations, before and after 4 weeks of treatment with vortioxetine, are presented in Table 3. The HAMD-17 and MADRS scores were significantly ($p<0.001$) decreased after 4 weeks of vortioxetine treatment compared to baseline scores. Plasma BDNF concentration was significantly ($p=0.018$) increased, while platelet 5-HT concentration was significantly ($p<0.001$) decreased after 4 weeks of treatment with vortioxetine compared to their corresponding baseline concentrations (Table 3).

Table 4 shows the clinical data (HAMD-17 and MADRS scores), and plasma BDNF and platelet 5-HT concentrations in patients with MDD, before and after 4 weeks of escitalopram treatment. The HAMD-17 and MADRS scores were significantly ($p<0.001$) lower 4 weeks after treatment with escitalopram compared to baseline scores. Plasma BDNF concentration did not differ significantly ($p=0.379$) before and after treatment with escitalopram. In contrast, platelet 5-HT concentration was significantly and greatly ($p<0.001$) reduced after 4 weeks of treatment with escitalopram compared to platelet 5-HT concentration before treatment (Table 4).

Prior to ANCOVA, log₁₀ data transformation of plasma BDNF and platelet 5-HT values was performed, due to their deviation from normal distribution. ANCOVA assessed the differences between post-treatment plasma BDNF and platelet 5-HT concentrations, in which the baseline values of plasma BDNF and platelet 5-HT concentration were used as covariates for the model correction. ANCOVA revealed a marginal trend toward significance regarding the difference in plasma BDNF concentration between patients receiving vortioxetine and escitalopram therapy for 4 weeks ($F(1,118)=4.58$; $p=0.034$), when the baseline plasma BDNF concentration was used as a covariate. These results indicate a greater vortioxetine-induced increase in plasma BDNF concentration compared to escitalopram-induced plasma BDNF concentration elevation. Significant difference in platelet 5-HT concentration was detected between patients receiving vortioxetine and escitalopram treatment for 4 weeks ($F(1,117)=26.86$; $p<0.001$, ANCOVA), when using baseline platelet 5-HT concentration as a covariate. These results reveal a significantly greater escitalopram-induced decrease in platelet 5-HT concentration compared to vortioxetine-induced reduction of platelet 5-HT concentration.

To evaluate whether pre-treatment plasma BDNF and platelet 5-HT concentrations might predict response to vortioxetine or escitalopram treatment, patients were subdivided into responders and non-responders, according to the reduction of HAMD-17 scores. In vortioxetine-treated responders and non-responders, no significant ($p>0.050$) differences in baseline plasma BDNF concentration or in the change of plasma BDNF were found (Table 5). No significant differences were observed for the baseline platelet 5-HT concentration and the change in platelet 5-HT concentration between vortioxetine-treated responders and non-responders (Table 5). Therefore, baseline plasma BDNF and platelet 5-HT concentrations could not predict treatment response to vortioxetine.

To predict response to escitalopram treatment, patients were subdivided into responders and non-responders. No significant ($p>0.050$) differences were found in the baseline plasma BDNF

concentration or changes in plasma BDNF concentration between responders and non-responders to escitalopram therapy (Table 6). In contrast, baseline platelet 5-HT concentration differed nominally ($p=0.026$; due to correction) and the change in platelet 5-HT concentration differed significantly ($p=0.015$) between responders and non-responders to escitalopram. Responders had higher baseline platelet 5-HT concentration and more pronounced change in platelet 5-HT than non-responders (Table 6). These results suggest that pre-treatment platelet 5-HT concentration and the change in platelet 5-HT could be used to predict treatment response to escitalopram.

4 Discussion

To the best of our knowledge, this longitudinal study is the first to explore and compare the effects of vortioxetine and escitalopram on plasma BDNF and platelet 5-HT concentration in patients with MDD. The results from the present study revealed that: 1) vortioxetine treatment for 4 weeks significantly increased plasma BDNF concentration, significantly decreased platelet 5-HT concentration, and significantly reduced HAMD-17 and MADRS scores; 2) escitalopram treatment for 4 weeks significantly decreased platelet 5-HT concentration, did not affect plasma BDNF concentration and significantly reduced HAMD-17 and MADRS scores; 3) vortioxetine treatment induced a significantly greater increase in plasma BDNF concentration compared to BDNF concentration after escitalopram treatment; 4) escitalopram treatment was associated with a significantly greater decrease in platelet 5-HT concentration than vortioxetine treatment; 5) baseline plasma BDNF and baseline platelet 5-HT concentrations could not predict treatment response to vortioxetine; 6) baseline platelet 5-HT concentration, but not baseline plasma BDNF concentration, was a good predictor of escitalopram response.

These different biological effects of vortioxetine and escitalopram are in line with numerous preclinical studies which demonstrated distinct and broader-range biological effects of vortioxetine compared to those of SSRIs (i.e., Waller et al., 2017; Franklin et al., 2018; Hlavacova et al., 2018), including escitalopram (Riga et al., 2017).

4.1 Different effects of vortioxetine and escitalopram on plasma BDNF levels

Vortioxetine and escitalopram showed similar efficacy in reducing symptoms of depression, as shown in the reduction of the HAMD-17 and MADRS scores, which is in agreement with previous trials (Jacobsen et al., 2015; Vieta et al., 2018). Our results confirmed well-known findings that both vortioxetine and escitalopram are highly effective antidepressants.

Treatment with vortioxetine increased while escitalopram did not affect plasma BDNF concentration in our MDD patients. Distinct effects between vortioxetine and escitalopram on plasma BDNF concentration might arise from their dissimilar activities on 5-HT system. The finding of the elevated plasma BDNF concentration after vortioxetine treatment confirms the results from our previous smaller study (Sagud et al., 2016), and is in line with the higher serum BDNF levels after one year of vortioxetine treatment compared to baseline values in patients with depressive disorder (Yan et al., 2019). Vortioxetine-induced elevation in plasma BDNF concentration might be attributed to the partial agonist activity of vortioxetine at 5HT1A and/or 5HT3 receptors (Bétry et al., 2015; Gonda et al., 2019), which is justified by the substantial evidence from animal models. Recent preclinical comparison of vortioxetine and escitalopram effect on extracellular levels of 5-HT and the desensitization of 5-HT1A receptor, suggested that the desensitization of 5-HT1AR with the inhibition of 5-HT3R contributed to the multimodal antidepressant action of vortioxetine in rats (Okada et al., 2019). Beneficial vortioxetine's effects on BDNF expression compared to SSRIs were detected in rats (Chen et al., 2018; Lu et al., 2018). In rats under basal condition, vortioxetine increased BDNF levels in hippocampus and number of synapses and mitochondria, in contrast to fluoxetine- and vehicle

treated rats (Chen et al., 2018). In rats exposed to chronic unpredictable mild stress, vortioxetine increased hippocampal BDNF levels, while fluoxetine had no such effects (Lu et al., 2018).

In line with a lack of effect of escitalopram on plasma BDNF concentration, treatment with escitalopram did not affect serum BDNF levels in patients with MDD (Ladea et al., 2013; Wolkowiz et al., 2011; Matrisciano et al., 2009; Table 7). No significant alteration of plasma BDNF concentration after escitalopram agrees with the lack of effect of paroxetine, mirtazapine, or venlafaxine on the in vitro BDNF production in the whole blood cell cultures in healthy volunteers (Lee et al., 2010). However, in contrast to our findings, escitalopram was reported to increase serum BDNF concentration in women with MDD (Aydemir et al., 2006) and in a small group of elderly depressed patients (Martocchia et al., 2014) when compared to healthy women or older controls (Table 7). Differences in the duration of treatment, diagnostic criteria, age and sex of the subjects might explain these distinct findings (Table 7).

Preclinical evidence supports the presumption that blood/plasma BDNF concentrations reflect brain BDNF levels, since plasma/blood and hippocampal BDNF levels were significantly correlated in pigs and rats (Klein et al., 2011). The assumption that the brain is the primary source of circulating BDNF has recently been questioned, since megakaryocytes represent the main source of BDNF (Chacón-Fernández et al., 2017). In turn, plasma BDNF, representing its free form (Serra-Millàs, 2016), is capable of crossing the blood-brain barrier (Podusslo and Curran, 1996; Pan et al., 1998). Therefore, although measuring BDNF in serum appears to be more reliable than in plasma (Zhou et al., 2017), plasma BDNF might be more closely related to brain BDNF levels.

Different effects on BDNF levels might also arise from some baseline differences between groups. Women have a tendency to show greater relative increases in plasma BDNF levels, while age was shown to have no effects, at least over the course of yoga and meditation retreat (Cahn et al., 2017). In our study plasma BDNF was not affected by age, gender and smoking either before or after treatment, and despite higher percentage of women in escitalopram group. In line with our data, BDNF level was not influenced by gender, age, family history, early onset, recurrence, or HAMD ratings in patients with MDD (Ming et al., 2019), or with smoking, gender and age in mentally healthy Caucasian subjects (Sustar et al., 2019).

4.2 Lack of correlation between plasma BDNF levels and treatment response

Despite different effects of vortioxetine and escitalopram on plasma BDNF concentration in our patients with MDD, the improvement of MDD symptoms was similar, and occurred independently of BDNF alterations. In line with previous studies (Matrisciano et al., 2009, Yoshimura et al., 2014), neither baseline plasma BDNF levels, nor the magnitude of their change, predicted endpoint response. No correlation between treatment response and plasma BDNF concentration (Table 7) was found in patients with panic disorder (He et al., 2019) or participants with MDD (Matrisciano et al., 2009) treated by escitalopram, or in MDD patients treated with citalopram (Haghigi et al., 2013), paroxetine or sertraline (Ming et al., 2019), and fluvoxamine (Yoshimura et al., 2014). Changes in depression scores were not related to modification of plasma BDNF concentration during paroxetine or venlafaxine treatment (Carboni et al., 2019). Likewise, baseline plasma BDNF concentration did not predict treatment response to paroxetine or venlafaxine (Carboni et al., 2019) or escitalopram (Brunoni et al., 2018) treatment (Table 7). In contrast to our results, depressed subjects with higher baseline serum BDNF levels achieved better therapeutic response to escitalopram, shown in decreased HAMD scores and clinical improvement (Wolkowiz et al., 2011). In addition, increased baseline serum BDNF levels were observed in responders compared to non-responders (Wolkowiz et al., 2011). Higher pre- and post-treatment plasma BDNF concentration were found in responders to fluoxetine or desvenlafaxine (Ghosh et al., 2015), while Kurita et al

(2012) reported higher initial plasma BDNF concentration in non-responders compared to remitters, but also higher endpoint plasma BDNF concentration in remitters. The discrepancies across studies might arise from the different follow-up times, and diversity of the type and doses of antidepressants (Table 7). Therefore, it might be speculated that plasma BDNF, while not being a potential marker of the early response to antidepressants, such as after 4 weeks, might be related to later antidepressant effects. It should be emphasized that some of these studies were underpowered (Kurita et al., 2012; Ghosh et al., 2015). The most recent meta-analysis, which included 20 studies of the antidepressant influence on peripheral BDNF concentration (but vortioxetine was not included), revealed that antidepressants elevated BDNF concentration only after 8 weeks, but not after 4 weeks of treatment (Zhou et al., 2017). When samples were stratified according to the medication, escitalopram did not change serum BDNF concentration at 4, 6, 8 and 12 weeks in total of 68 depressed patients (Zhou et al., 2017). However, patients treated with citalopram, the racemic mixture of R(-)- and S(+)-enantiomers (escitalopram), during 4 weeks, demonstrated increase in post-treatment plasma BDNF concentration, but the citalopram dose was twice as high than the minimal dose (Haghigi et al., 2013). In contrast, our patients were treated with 10 mg escitalopram, which represents its lowest therapeutic dose. Although this minimal escitalopram dose has markedly decreased platelet 5-HT concentration, suggesting its strong inhibition of SERT, it cannot be excluded that higher escitalopram dose might have also increased plasma BDNF concentration. Likewise, escitalopram did not affect plasma BDNF concentration during 3-week treatment with 10 mg dose, or with 20 mg dose during next seven weeks (Brunoni et al., 2018).

In addition, while patients in our and other (Haghigi et al., 2013) study were either drug-naïve or drug-free, participants from the Kurita study (2012) were treated with various antidepressants at baseline, which might have affected pre-treatment BDNF concentration. Besides possible influence of the prior antidepressant use, the differences in plasma BDNF concentration across studies might be explained by the different protocols of plasma isolation and centrifugations, since BDNF concentration in normal plasma is higher than in platelet-poor plasma (Gejl et al., 2019).

4.3 Different effects of vortioxetine and escitalopram on platelet 5-HT concentration

The marked decrease of platelet 5-HT content in our patients treated with escitalopram is consistent with other reports describing association between SSRI treatment and platelet 5-HT (Table 7). In patients treated with vortioxetine, significant reduction of platelet 5-HT concentration was detected, confirming our previous results (Sagud et al., 2016), but with the enlarged sample. However, this fall of platelet 5-HT after vortioxetine treatment was significantly smaller than escitalopram-induced reduction of platelet 5-HT content. As far as we are aware, there are no other data on the effect of vortioxetine and comparison with the effects of escitalopram on platelet 5-HT concentration in patients with MDD. The effects of escitalopram and vortioxetine on platelet count were evaluated after 4 weeks of treatment in drug-naïve depressed patients, and escitalopram significantly, while vortioxetine marginally reduced platelet count (Song et al., 2012). Although in our study escitalopram elicited greater fall of platelet 5-HT than vortioxetine, in line with the escitalopram-induced greater reduction of the platelet count (Song et al., 2012), we controlled for this possible effect, since platelet 5-HT was corrected for the platelet protein concentration. Since SSRIs affect 5-HT levels similarly in platelets and neurons (Yubero-Lahoz et al., 2013), increased central SERT occupancy would be associated with decreased platelet 5-HT concentration. Reduced platelet 5-HT concentration in our depressed patients treated with escitalopram and vortioxetine suggests that vortioxetine was able to inhibit (although to a lesser degree than escitalopram) platelet SERT. Slightly different results were reported in healthy subjects, since vortioxetine and paroxetine similarly affected SERT occupancy (Wilson et al., 2015). These two

medications similarly inhibited SERT in tryptophan-depletion model of depression in rats, but vortioxetine significantly reversed fall of pineal melatonin and 5-HT while paroxetine was without effect (Franklin et al., 2018). These data suggest that similar SERT occupancy might not be associated with similar biochemical and/or clinical effects. The discrepancies might be explained with the different vortioxetine doses and inclusion of cases vs. controls. Of note, healthy participants (Wilson et al., 2015) received vortioxetine dose of 20 or 40 mg daily, which is maximal and twice the maximal dose, respectively, whereas paroxetine dose was 20 mg, representing the lower end of its therapeutic range. On the contrary, our patients were treated by 10 mg of vortioxetine, which was reported to exert about 53% of SERT blockade in the raphe nuclei, in contrast to 60 mg daily dose which occupied 98% of SERT at steady-state levels in healthy males (Stenkrona et al., 2013). Therefore, our results have demonstrated that the partial SERT blockade induced by 10 mg vortioxetine dose (Stenkrona et al., 2013), might also be reflected by the partial reduction of platelet 5-HT concentration, unlike the effects of escitalopram, which produced 85% SERT blockade in the same brain region (Baldinger et al., 2014), and induced great platelet 5-HT fall.

4.4 Platelet 5-HT levels and treatment response

Our responders to escitalopram had higher baseline platelet 5-HT concentration and greater change of platelet 5-HT concentration than non-responders, which is in line with previous reports in patients treated with fluoxetine (Castrogiovanni et al., 2003). Platelet 5-HT concentration before treatment and the change in platelet 5-HT was significantly associated with treatment response to escitalopram. Similarly, platelet 5-HT content correlated with depression severity measured by HAMD scale following 4-week treatment with fluoxetine or paroxetine (Zhuang et al., 2018). However, our results disagree with similar reduction in platelet 5-HT regardless of the response status to paroxetine (Figueras et al., 1999; Muck-Seler et al., 2002) or sertraline (Pivac et al., 2003). Differences across studies might be explained by the smaller number of patients included in the previous studies, and different SSRIs used (Table 7). Such results might reflect potentially lower escitalopram levels in non-responders, given that the decrease in platelet 5-HT content by SSRIs was dose-related (Maurer-Spurej et al., 2004). However, paroxetine concentration did not correlate with either post-treatment platelet 5-HT concentration, or the magnitude of their change (Figueras et al., 1999). Our finding of the higher baseline 5-HT concentration in responders to escitalopram is in contrast with higher pre-treatment 5-HT content in non-responders to paroxetine (Figueras et al., 1999). The discrepancies might arise from the methodological differences, such as definitions of response and characteristics of depression. In the present trial treatment response was defined as $\geq 50\%$ reduction from the baseline HAMD-17 scores, while Figueras et al. (1999) considered response as the reduction of HAMD score below 9. Platelet 5-HT concentration might vary according to MDD characteristics. Namely, platelet 5-HT was increased in depression with psychotic features (Pivac et al., 1997), but decreased in suicidal (Pivac et al., 1997; Roggenbach et al., 2007) and melancholic patients (Figueras et al., 1999). Of note, higher platelet 5-HT content and uptake capacity was reported in drug-free patients with depression or anxiety, compared to healthy subjects (Zhuang et al., 2018). Therefore, responders to escitalopram might also have had higher SERT capacity than non-responders. In agreement, in preclinical model, a higher hippocampal SERT capacity was associated with greater sensitivity to fluoxetine (Tang et al., 2014).

4.5 Limitations

The study was not double-blind, although measurements of the biochemical data were done blind to treatment allocation. Although the study excluded psychotic patients, we did not distinguish the other subtypes of depression. The period of escitalopram treatment of 4 weeks might be too short to affect BDNF concentration, given its sequential increase up to week 12

of treatment (Zhou et al., 2017; Ming et al., 2019). However, the 4-week period was sufficient to elevate BDNF concentration by vortioxetine. In addition, physical activity was not recorded, and, while peripheral BDNF concentration, including those measured in plasma, have increased immediately after a single exercise, at least in male college students (Cho et al., 2012), it cannot be excluded that patients receiving vortioxetine had sharper increase in physical activity compared to those treated with escitalopram. Since both groups had similar improvement of depression, drastic changes in physical activity levels in these patients are not realistic. Concentration of antidepressants was not measured. Moreover, BDNF polymorphism, such as Val66Met, which might also influence plasma BDNF levels, as reported by some (Colle et al., 2017) but not all (Ming et al., 2019; Sustar et al., 2019) authors, was also not determined.

5 Conclusion

In the present trial vortioxetine robustly increased plasma BDNF concentration in contrast to escitalopram, while both drugs decreased platelet 5-HT concentration. Baseline platelet 5-HT concentration was a good predictor of escitalopram response. These findings might be due to vortioxetine unique mechanism of action, but the clinical implications are unclear, given that 1) both drugs similarly improved symptoms of depression, and 2) this improvement was unrelated to either baseline or posttreatment BDNF levels. It remains to be determined whether this finding extends during long-term vortioxetine treatment, and which, if any, clinical effects emerge from BDNF increase.

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Author contributions

MS and NP were responsible for the idea and design of the study protocol. AD, MS, AMP, BVC, ZK, MZ, SJ were involved in the conduct of the research (collection of data, patient recruitment, inclusion/exclusion criteria), and sampling of the patients. AD, MS, AMP, BVC, ZK, MZ, SJ explained the research goals and described protocol in details to the patients; insured participant adherence for the participation in the study, motivated, selected, diagnosed, and evaluated patients and did psychiatric diagnoses and psychological analyses. MNP, GNE, DSS, LT and MK isolated plasma and platelets and determined platelet serotonin. MNP determined plasma BDNF and did the data analysis and statistical analysis and was responsible for the databases. All authors were included in the interpretation of findings, and preparation of the first draft of the manuscript. MS and NP wrote the first draft of the manuscript. All authors have read and approved the final version and have contributed substantially to the design, performance, analysis, and reporting of this study.

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Table 1. Study procedures.

Assessment	Baseline	Week 1	Week 4
Structured clinical interview, clinical and demographic data	x		
HAMD-17, MADRS	x	x	x
Platelet 5-HT and plasma BDNF concentration	x		x

HAMD-17: The Hamilton Depression Rating Scale-17; MADRS: Montgomery-Åsberg Depression Rating Scale

Table 2. Demographic and clinical data of patients with major depressive disorder.

Demographic and clinical parameters	Vortioxetine N=61	Escitalopram N=60	Test statistics
Sex (male/female)	27/34	14/46	$X^2=5.91$; $p=0.015$
Smoking (yes/no)	22/39	20/40	$X^2=0.10$; $p=0.752$
Depressive episode (first or single/recurrent)	33/28	46/14	$X^2=6.80$; $p=0.009$
Family history of depression (yes/no)	12/48	24/36	$X^2=5.71$; $p=0.017$
Number of suicide attempts	0 (0-2)	0 (0-1)	$U=1803.0$; $p=0.395$
Age (years)	52 (18-78)	45.5 (19-67)	$U=1562.0$; $p=0.164$
Duration of illness (months)	0 (0-420)	0 (0-192)	$U=1397.5$; $p=0.019$
Number of depressive episodes	0 (0-10)	0 (0-10)	$U=1372.0$; $p=0.017$
HAMD-17 score (baseline)	23 (17-36)	21 (17-37)	$U=1388.5$; $p=0.022$
MADRS score (baseline)	27 (16-51)	24 (17-41)	$U=1288.5$; $p=0.006$

Categorical data was analysed with Chi-square test ($df=1$). Numerical data was analysed with Mann-Whitney U test and shown as median (range). HAMD-17: The Hamilton Depression Rating Scale-17; MADRS: Montgomery-Åsberg Depression Rating Scale; N: number of subjects.

Table 3. Comparison of clinical parameters (HAMD-17 and MADRS scores), and plasma BDNF and platelet 5-HT concentrations before and after 4 weeks of treatment with vortioxetine.

Vortioxetine	At baseline			After treatment			Wilcoxon signed-rank test	
	Mean	Percentile		Mean	Percentile		Z	p
	(min-max)	25th	75th	(min-max)	25th	75th		
HAMD	23 (17-36)	20	28	10 (1-26)	5	14	5.0	<0.001
MADRS	27 (16-51)	25	32	11 (0-35)	5	17	11.0	<0.001
Plasma BDNF (ng/ml)	0.326 (0.028-1.103)	0.203	0.574	0.445 (0.054-3.196)	0.221	0.793	1275.5	0.018
Platelet 5-HT (nmol/mg proteins)	1.18 (0.13-3.72)	0.79	1.16	0.62 (0.22-1.86)	0.39	0.88	168.5	<0.001

5-HT: serotonin; BDNF: Brain-Derived Neurotrophic Factor; HAMD: The Hamilton Depression Rating Scale-17; MADRS: Montgomery-Åsberg Depression Rating Scale

Table 4. Comparison of clinical parameters (HAMD-17 and MADRS scores), and plasma BDNF and platelet 5-HT concentrations before and after 4 weeks of treatment with escitalopram.

Escitalopram	At baseline			After treatment			Wilcoxon signed-rank test	
	Mean	Percentile		Mean	Percentile		Z	p
	(min-max)	25th	75th	(min-max)	25th	75th		
HAMD	21 (17-37)	19	24	8.5 (1-34)	5	16	8.0	<0.001
MADRS	24 (17-41)	21	29	10 (1-46)	4.5	19.5	14.0	<0.001
Plasma BDNF (ng/ml)	0.342 (0.026-1.574)	0.218	0.506	0.330 (0.067-1.392)	0.180	0.582	795.5	0.379
Platelet 5-HT (nmol/mg proteins)	1.30 (0.19-3.38)	0.99	1.65	0.30 (0.04-3.10)	0.16	0.66	75.0	<0.001

5-HT: serotonin; BDNF: Brain-Derived Neurotrophic Factor; HAMD: The Hamilton Depression Rating Scale-17; MADRS: Montgomery-Åsberg Depression Rating Scale

Table 5. Comparison of baseline concentrations and changes (Δ) in plasma BDNF and platelet 5-HT concentrations between responders and non-responders to vortioxetine therapy (determined according to the reduction of HAMD-17 scores).

Vortioxetine	Non-responders			Responders			Mann-Whitney U test	
	Mean (min-max)	Percentile		Mean (min-max)	Percentile		U	p
		25th	75th		25th	75th		
Plasma BDNF baseline (ng/ml)	0.322 (0.040-1.103)	0.189	0.484	0.418 (0.028-1.010)	0.220	0.591	468.0	0.373
Δ plasma BDNF (%)	39.62 (-64.92-715.35)	-26.02	139.79	25.29 (-77.41-810.34)	-25.51	69.52	387.0	0.724
Platelet 5-HT baseline (nmol/mg proteins)	1.22 (0.13-3.07)	0.86	1.96	1.12 (0.16-3.72)	0.77	1.64	370.0	0.539
Δ platelet 5-HT (%)	-48.27 (-86.97-153.85)	-56.75	-16.43	-47.10 (-83.54-443.75)	-63.36	-31.82	378.0	0.623

5-HT: serotonin; BDNF: Brain-Derived Neurotrophic Factor

Table 6. Comparison of baseline concentrations and changes (Δ) in plasma BDNF and platelet 5-HT concentrations between responders and non-responders to escitalopram therapy (determined according to the reduction of HAMD-17 scores).

Escitalopram	Non-responders			Responders			Mann-Whitney U test	
	Mean (min-max)	Percentile		Mean (min-max)	Percentile		U	p
		25th	75th		25th	75th		
Plasma BDNF baseline (ng/ml)	0.338 (0.026-1.220)	0.213	0.492	0.367 (0.075-1.574)	0.223	0.565	469.0	0.577
Δ plasma BDNF (%)	-7.73 (-71.13-373.39)	-36.33	30.71	-5.23 (-84.53-550.67)	-37.54	43.68	420.0	0.856
Platelet 5-HT baseline (nmol/mg proteins)	1.12 (0.19-1.74)	0.74	1.44	1.32 (0.31-3.38)	1.09	1.86	580.0	0.026
Δ platelet 5-HT (%)	-56.82 (-95.24-136.64)	-82.08	-25.91	-81.49 (-95.35-2.42)	-89.50	63.03	271.5	0.015

5-HT: serotonin; BDNF: Brain-Derived Neurotrophic Factor

Table 7. Short overview of the original research articles cited in the text in which the effect of antidepressant therapy on BDNF and 5-HT levels was investigated.

Study	Antidepressant	Dose/day	Treatment duration	Subjects	Sample type	Diagnosis	Parameter tested	Treatment effect
Aydemir et al., 2006	Escitalopram	10 mg	6 wk	H	Serum	D	BDNF	↑ BDNF
Brunoni et al., 2018	Escitalopram	10-20 mg	3 wk, 10 wk	H	Plasma	D	NGF, BDNF, GDNF, IL-1 β , IL-6, IL-8, IL-10, IL-12p70, IL-18, IL-33, TNF- α , sTNFr1, sTNFr2	NS
Carboni et al., 2019	Paroxetine	Flexible-dose study	10 wk	H	Plasma	D	IL-6, IL-10, TNF- α , TNFr2, BDNF, CRP, MMP9, PAI1	↑ TNF- α , IL-6, IL-10, CRP
	Venlafaxine						NS	
Castrogiovanni et al., 2003	Fluoxetine	20 mg	30 d	H	Plasma Platelets	D	5-HT	↓ platelet 5-HT, ↑ plasma 5-HT
Chen et al., 2018	Vortioxetine	1.6 g/kg food	7 d	R	HPC		BDNF	↑ BDNF
	Fluoxetine	160 mg/L water						NS

Study	Antidepressant	Dose/day	Treatment duration	Subjects	Sample type	Diagnosis	Parameter tested	Treatment effect
	Sertraline	25–100 mg						
	Sulpiride	300 mg						
	Trazodone	50 mg						
Ladea et al., 2013	Escitalopram	10-20 mg	4 wk, 12 wk, 24 wk	H	Serum	D	BDNF	↑ BDNF
Lee et al., 2010	Amitriptyline	10 ng/ml, 100 ng/ml, 1 kg/ml	48 h	H	Whole blood cell culture	HC	BDNF	↑ BDNF production
	Paroxetine	5, 50, 500 ng/ml						NS
	Mirtazapine	5, 50, 500 ng/ml						NS
	Venlafaxine	15 ng/ml, 150 ng/ml, and 1.5 kg/ml						NS
Lu et al., 2018	Vortioxetine	7.2 mg/kg food	3 wk	R	Hippocampus		BDNF	↑ BDNF
	Fluoxetine	14.4 mg/kg food						NS
Martocchia et al., 2014	Escitalopram	10 mg	2 mos	H	Serum	D	BDNF	↑ BDNF
Matrisciano et al., 2009	Sertraline	50–200 mg	5 wk, 6 mos	H	Serum	D	BDNF	↑ BDNF (5 wk and 6 mos)
	Escitalopram	75–225 mg						NS
	Venlafaxine	10–20 mg						↑ BDNF (6 mos)
Maurer-Spurej et al., 2004	Citalopram	10, 20, 30, 50, 64 mg	≥ 6 wk	H	Platelets	D	5-HT	↓ platelet 5-HT
	Paroxetine	40 or 60 mg						
	Fluoxetine	20, 30 or 40 mg						
	Sertraline	250 mg						
	Fluvoxamine	300 mg						
	Fluoxetine	Not reported		H	Plasma	D	BDNF	

Study	Antidepressant	Dose/day	Treatment duration	Subjects	Sample type	Diagnosis	Parameter tested	Treatment effect
Ming et al., 2019	Paroxetine		4 wk, 8 wk, 12 wk					↑ BDNF (12 weeks of treatment)
Muck-Seler et al., 2002	Tianeptine	37.5 mg	4 wk	H	Plasma Serum Platelets	D	platelet and serum 5-HT, platelet MAO activity, plasma cortisol, PRL	NS
	Paroxetine	20 mg						↓ platelet 5-HT
Pivac et al., 2003	Sertraline	42.5 mg (maximum 100 mg)	4 wk, 24 wk	H	Platelets	D	platelet 5-HT, platelet MAO activity	↓ platelet 5-HT (4 and 24 wk) ↓ platelet MAO activity (24 wk)
Sagud et al., 2016	Vortioxetine	5-15 mg	4 wk	H	Plasma Platelets	D	BDNF, platelet 5-HT	↓ platelet 5-HT ↑ BDNF
Wolkowiz et al., 2011	Escitalopram	10 mg (4 wk) + 20 mg (4 wk)	8 wk	H	Serum	D	BDNF	↑ BDNF
Yan et al., 2019	Sertraline	50-200 mg						
	Vortioxetine + CBT	10 mg + 2 sessions/wk	8 wk	H	Serum	D	BDNF	↑ BDNF
Yoshimura et al., 2014	Paroxetine Sertraline	30.5±12.4 mg 76.7±24.0 mg	4 wk, 8 wk	H	Serum	D	BDNF	NS

Study	Antidepressant	Dose/day	Treatment duration	Subjects	Sample type	Diagnosis	Parameter tested	Treatment effect
	Fluvoxamine	100±26.3 mg						
Zhuang et al., 2018	Escitalopram Fluoxetine Paroxetine Sertraline	5 mg 20-40 mg 20-40 mg 50 mg	4 wk	H	Platelets	D	platelet 5-HT, capacity of SERT	↓ platelet 5-HT

5-HT: serotonin; BDNF: brain-derived neurotrophic factor; CBT: cognitive-behavioural therapy; CRP: C-reactive protein; d: day; D: depression; ECT: electroconvulsive therapy; GDNF: glial-cell line derived neurotrophic factor; HC: healthy control; HPC: hippocampus; IL: interleukin; MAO: monoamine oxidase; MMP9: matrix metalloproteinase 9; NA: noradrenaline; NS: no effect; NGF: nerve growth factor; PAI1: plasminogen activator inhibitor-1; PD: panic disorder; PRL: prolactin; SERT: serotonin transporter; TNF- α : tumor necrosis factor-alpha; TNFr: TNF- α receptor; wk: week.