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# VEGF serum levels in depressed patients during SSRI antidepressant treatment

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ABSTRACT

Recent evidence indicates that the vascular endothelial growth factor (VEGF) may be involved in the neuronal mechanisms underlying both the depression aetiology and the response to pharmacological and non pharmacological antidepressant treatments. To investigate whether VEGF peripheral levels are altered in depression and are modulated by antidepressant therapies, we analyzed the serum VEGF concentrations in 25 subjects affected by major depression (MD) before (T0) and after 8 (T8) and 12 (T12) weeks of escitalopram treatment. No significant alterations in VEGF serum levels were found at T0, even considering possible effects of confounders such as gender and smoking habit ($r^2$=0.227 $p$=0.74). No changes appeared during the treatment ($F$(1.83, 43.86)=0.962; $p$=0.383) and there was no correlation between percentage VEGF variations at T12 and symptoms improvements ($p$=0.823).
The present work represents the first report on the evaluation of serum VEGF levels in MD patients. However, before discarding serum VEGF as a biochemical marker in the diagnosis and treatment of depression, our negative results need to be confirmed in larger patient samples stratified for clinical characteristics, co-morbidities, cardiovascular diseases and confounding factors.



## 1. Introduction

The vascular endothelial growth factor (VEGF) is an angiogenic cytokine able to induce vasopermeability in many types of tissues (Ferrara et al., 2003), including the Blood Brain Barrier (Góra-Kupilas and Jośko, 2005; Rigau et al., 2007) and to facilitate the neurogenesis and proliferation of neurons in the adult hippocampus (Palmer et al., 2000). Recent evidence indicates that VEGF can act as a neuroprotective factor in the adult brain, inhibiting apoptosis and inducing growth of the associated vascular-neuronal networks (Brockington et al., 2004; Raab and Plate, 2007). VEGF influences synaptic plasticity in hippocampus-dependent processes, such as learning and memory (Cao et al., 2004), and modulates synaptic transmission (McCloskey et al., 2005). These actions are important in the behavioural response of animal models that are receptive to multiple classes of antidepressants, and thereby serve as valuable predictors of the antidepressant's activity (Martin et al., 1986; Martin et al., 1987; Chen et al., 2001; Sherman et al., 1982).

There is increasing evidence of a co-morbidity between major depression (MD) and cardiovascular disease: different studies indicate that depressive symptoms increase the risk of cardiovascular diseases, although this may reflect reversed causality (Baune et al., 2006; Kammphuis et al., 2006; Rugulies, 2002). Recently, Dome et al. (2008) found decreased numbers of circulating endothelial progenitor cells in patients with a current episode of MD. Such a decrease is considered a sign of alteration of the vascular function in a variety of cardiovascular risk conditions. However, the biological mechanisms by which depression may increase the risk of cardiovascular events have not been completely clarified. Because of its wide-range action, VEGF could represent a molecular link between cardiovascular diseases and major depression, which might share common risk factors.

Moreover, it is now clear that VEGF can contribute to the long-term adaptations required for the action of antidepressants (Tanis et al., 2007; Duman and Monteggia, 2006). In fact, preclinical studies indicate that antidepressant drugs induce an expression of VEGF that follows the time course of the drugs' therapeutic effectiveness (Warner-Schmidt and Duman, 2007), similarly to electroconvulsive shock (ECS) (Newton et al., 2003; Altar et al., 2004).

*Abbreviations:* BMI, Body Max Index; BDNF, brain-derived neurotrophic factor; HDRS, Hamilton Depression Rating Scale; MD, major depression; VEGF, vascular endothelial growth factor.

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Recently, higher expression levels of VEGF mRNA were found in peripheral leukocytes of depressed patients. These levels began to decrease after a treatment with antidepressants was initiated, and the decrease's time course paralleled the clinical improvement (Iga et al., 2007). On the contrary, no significant difference in VEGF plasma levels was detected between treated MD patients and healthy controls (Dome et al., 2008). Even though higher VEGF serum levels have been associated to a depressive state in patients affected by cancer (Sharma et al., 2007; Lutgendorf et al., 2002), to date no studies have been conducted in depressed patients before and after treatment with antidepressants.

On these bases, the aim of this study was to investigate putative alterations in VEGF serum levels in MD patients as compared to control subjects. A second aim was the assessment of longitudinal changes in VEGF serum content, hypothesized to be induced by a 12-week long antidepressant treatment with escitalopram.

## 2. Methods

### 2.1. Subjects and escitalopram treatment

Twenty-five in- and outpatients (20 females, 5 males; mean age 43.36±9.97 years) were recruited in the Biological Psychiatry Unit of IRCCS Centro 'S. Giovanni di Dio' FBF, Brescia, Italy. Inclusion criteria were: at least moderate severity of depression according to either ICD 10 or DSM IV criteria, age 18–65 years, Caucasoid European parents. Exclusion criteria were: a personal history of bipolar affective disorder, schizophrenia, mood incongruent psychotic symptoms, primary substance abuse or primary organic disease, current or actively seeking pregnancy, current treatment with antidepressants, antipsychotics, mood stabilizers, or any regular treatment for a medical condition (including hypercholesterolemia, hypertension), first-degree relatives with a history of bipolar affective disorder or schizophrenia. All patients underwent a complete clinical evaluation (general physical examination, including the recording of blood-pressure, height and body weight) and routine laboratory examinations at the time of enrolment (T0). Subjects showing hypertension, signs of infections, obesity or blood values outside normal ranges were excluded. Mean Body Max Index (BMI) was 24.09±3.12 and 17 patients were non-smokers while 8 were smokers.

Before entering the study, the wash-out period (low doses of benzodiazepines were allowed) was at least 2 weeks for 13 patients and 1 week for 5, while 7 patients were at their first episode of depression. All patients were treated with escitalopram for a period of three months, the mean drug dosage at T8 was 15.8±4.49 mg/day. The 21-item Hamilton Depression Rating Scale (HDRS) was used at the beginning and during the treatment to assess both baseline (T0) severity of illness and symptoms amelioration. Average baseline severity of illness (HDRS, T0) was 19.68±2.76.

Biological sampling and clinical evaluations were performed in the morning before the treatment's start (T0), 8 weeks (T8) and 12 weeks (T12) into the treatment. Changes in the severity of illness were assessed by percentage decrease in HDRS scores at T8 and T12.

A control group (C) of 30 subjects (25 females, 5 males; mean age 41.57±8.26 years) with no history of DSM-IV-TR Axis I disorders (confirmed by MINI interview) and no family history of psychoses and mood disorders was enrolled in the study. Exclusion criteria for control subjects were the same as for depressed patients. Mean Body Max Index (BMI) was 22.81±3.06 and 25 were non-smokers, while 5 subjects were smokers. No differences in demographic and clinical characteristics were observed between patients and controls.

The study was approved by the local Ethical Committee, and all subjects enrolled gave their informed consent to the participation.

### 2.2. Serum VEGF determination

Venous blood samples were collected from patients and controls in the morning (between 8.00 and 9.00 a.m.) after an overnight fast in anticoagulant-free tubes. They were kept at room temperature for 2 h, followed by 1 h at 4 °C before serum separation by centrifugation (3000 rpm for 15 min at 4 °C). Serum samples were stored at −80 °C till the time of assay. VEGF levels were measured by an ELISA method using the human VEGF Quantikine kit (R&D system, Minneapolis, USA), following the manufacturer's instructions. Inter-assay variances were below 8%. All analyses were conducted in duplicate and VEGF contents were expressed as equivalent of human recombinant proteins. The detection limit of the assay was 9 pg/ml and data were expressed as pg of protein/ml of serum.

### 2.3. Statistical analysis

Demographic and clinical characteristics in our patient and control samples were described either in terms of mean±SD if quantitative, or in terms of proportions. After checking for normality, student *t* tests were used when appropriate to evaluate differences in quantitative variables. Qualitative variables were tested by means of $\chi^2$ and Fisher tests. Pearson coefficient was used to evaluate bivariate correlations.

Regression analysis of variance was used to examine the simultaneous contribution of significant covariates and their interactions on VEGF peripheral levels.

Clinical and biological changes occurring during drug treatment were analysed by means of a General Linear Model, in a repeated measures design with Time (T0, T8, T12) as within-subjects factor. Greenhouse–Geisser correction was applied to the degrees of freedom when the assumption sphericity was violated. The SPSS, version 13.0, software package (http://www.spss.com) was used for all statistical calculations.

## 3. Results

The drug treatment improved depression symptomatology as measured with HDRS (score mean values±standard deviations: T0=19.68±2.76; T8=10.44±6.31; T12=6.68±3.73): ANOVA with Time (T0, T1 and T2) as within-subjects factor, delivered HDRS scores significantly decreased during escitalopram treatment [HDRS Greenhouse–Geisser (G-G) correction $F(1.56, 37.52)=87.31$ $p<0.001$], with a medium percentage improvement at T12 of 66.15% (%HDRS). In particular, planned "repeated" contrasts indicated a significant decrease in HDRS scores between T0 and T8 [$F(1, 24)=54.81$ $p<0.001$] and between T0 and T12 [$F(1, 24)=251.00$, $p<0.001$].

No significant differences in VEGF serum levels were evidenced between drug-free depressed patients and controls (T0: 403.8±243.4 pg/ml, C: 405.0±215.6 pg/ml, $p=0.984$) (Fig. 1). A significant effect of gender ($p=0.004$) and smoking habit ($p=0.005$) on serum VEGF content was observed in the whole sample while no correlations were found between serum VEGF and either age ($p=1.00$) or BMI ($p=0.26$). Also, no effect of the diagnostic status (T0 vs. C, $p=0.74$) on serum VEGF was evidenced in multiple linear regression ($r^2=0.227$), taking into account the effects of covariate gender ($p=0.025$) and smoke ($p=0.023$). A significant correlation was found between VEGF serum levels in the patient group and blood platelet count ($r=0.542$, $p=0.005$).

No changes in serum VEGF were evidenced during the treatment [serum VEGF at T0=403.8±243.4 pg/ml, at T8=409.0±273.7, at T12=426.3±262.8; ANOVA with time G-G correction: $F(1.83, 43.86)=0.962$; $p=0.383$; Fig. 1]. No modulation operated by the drug was evident in the subsample of responder patients (defined as %HDRS reduction>50%) at T8 [$n=13$, serum VEGF at T0=367.2±234.2 pg/ml, at T8=344.5±221.3, at T12=377.8±255.2; ANOVA with time G-G correction: $F(1.88, 22.66)=1.37$; $p=0.274$] and at T12 [$n=21$, serum VEGF at T0=400.26±252.7 pg/ml, at T8=396.5±265.9, at T12=413.3±258.3; ANOVA with time G-G correction: $F(1.96, 39.28)=0.510$; $p=0.601$]. Finally, no correlations were found between percentage