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Vascular endothelial growth factor A gene expression level is higher in patients with major depressive disorder and not affected by cigarette smoking, hyperlipidemia or treatment with statins

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Elevated levels of vascular endothelial growth factor (VEGF) are observed in conditions with vessel and neuron damage or pathological arborization and can therefore be detected in chronic inflammatory process, cardiovascular disease and depression. Hyperlipidemia and cigarette smoking are two factors that have been implicated in endothelial damage. The high comorbidity between cardiovascular disease and major depression disorder (MDD) prompted us to study the effect of cigarette smoking, hyperlipidemia and statin treatment on the *VEGFA* mRNA and protein expression levels measured in MDD patients. We analyzed 38 MDD patients and 38 healthy control individuals and observed that the MDD group had a significantly higher *VEGFA* mRNA level and protein serum concentration (P=0.001; P<0.001, respectively). We found no significant association between *VEGFA* expression at the mRNA or protein level and cigarette smoking, hyperlipidemia or treatment with statins (P>0.05). Interestingly, patients who had attempted suicide had a lower VEGF serum level compared with patients who had not attempted suicide. The translational value of this finding remains unknown. A higher VEGF concentration may play a potentially significant role in the pathogenesis of depression, and the expression level appears to be unaffected by additional factors.

Key words: major depressive disorder, vascular endothelial growth factor, cigarette smoking, hyperlipidemia

INTRODUCTION

Vascular endothelial growth factor (VEGF) is a neurotrophic and angiogenic mitogen that is necessary for proper embryonic and adult human growth and development. Virgintino and coworkers (2003) showed that the VEGF protein is present in multiple cell types in the fetal human brain. Some of these cell types include cells proper to the nervous tissue, such as neuroepithelial cells, neuroblasts and radial glia cells, and non-neuronal cells, such as endothelial and periendothelial cells. These authors suggested that the VEGF amount in these cells is developmentally regulated and correlated with angiogenesis, which is responsive to the high metabolic demands of the differentiating neocortex. The VEGF level is also higher under specific pathological conditions related to neuron and vessel repair as well as their uncontrolled arborization. VEGF is main factor involved in regulating the neoangiogenesis process and therefore, it is a target of novel anticancer therapy research. The elevated VEGF level in tumor tissue may result from the hypoxic environment in the rapidly growing tumor, which may be an adaptive mechanism for providing the tumor with oxygen and nutrients and maintaining growth and metastases (Gacche and Meshram 2013). The *VEGFA* expression level is higher in inflammatory disorders such as sepsis (Chancharoenthana et al. 2013), rheumatoid arthri-

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tis (Zhang et al. 2013), and psoriatic arthritis (Yamamoto 2013). According to the most recent studies, depression is also considered as an inflammatory disorder (Anisman 2011).

An elevated *VEGFA* expression level has also been reported in cardiovascular disease (Jin et al. 2000, Sun et al. 2003). Furthermore, it is necessary to mention, that the underlying cause of cardiovascular disorder is atherosclerosis, which is a slowly progressing chronic disorder with an established inflammatory background (Woolard 2013). It was found that VEGF exerts a neurotrophic and neuroprotective effect under hypoxic conditions, which are similar to the effect observed in cerebral insult. Using an *in vitro* cerebral insult model, Jin and others (2000) demonstrated that VEGF has a direct neuroprotective effect in ischemic tissue. In HN33 cells, an immortalized hippocampal neuronal cell line, VEGF approximately doubled the number of cells that survived 24 h of hypoxia and glucose deprivation. Sun and colleagues (2003) produced focal cerebral ischemia using middle cerebral artery occlusion for 90 minutes in the adult rat brain and found that VEGF has an acute neuroprotective effect and longer latency effects on new neurons survival and angiogenesis in the ischemic brain. The probable mechanism underlying this effect is that cerebral ischemia triggers hypoxia, a sensing mechanism that activates the transcription factor hypoxia-inducible factor – 1, which induces *VEGFA* expression (Bergeron et al. 1999). A few studies have investigated the potential correlation between cigarette smoking, a factor known to contribute to endothelial damage, and the VEGF serum concentration in healthy smokers and have shown that there appears to be no significant difference compared with nonsmokers (Belgore et al 2000, Schmidt-Lucke et al 2005).

Interestingly, it has been shown that the *VEGFA* mRNA expression level (Iga et al. 2007, Kahl et al. 2009) and serum VEGF protein concentration (Lee and Kim 2012) are higher in depressed patients compared with healthy individuals. It has been suggested that this increase in expression may be a repair response to the hippocampal neural damage that underlies the pathogenesis of depression (Blumberg et al. 2008). There are conflicting results on the effect of antidepressant treatments on the *VEGFA* expression level. Iga and coauthors (2007) reported that the *VEGFA* expression level decrease after a successful antidepressant treatment. Ventriglia and others (2009), however, found that an escitalopram treatment had no effect on the VEGF serum levels.

The high comorbidity of MDD and cardiovascular disorder (Elderon and Whooley 2013) prompted us to investigate whether the *VEGFA* expression level in MDD patients is affected by two factors known to cause endothelial lesions, hyperlipidemia and cigarette smoking. To the best of our knowledge this is the first study reported that investigates this issue in MDD patients. Furthermore, we also investigated the effect of statin treatment on *VEGFA* expression. Giurgea and colleagues (2006) found that simvastatin treatment can result in a significant decrease in the VEGF serum levels in hypercholesterolemic patients.

In the present study, we first determined whether there is a difference in the *VEGFA* expression level between the MDD group and control group. We then investigated the potential correlations between the *VEGFA* mRNA and protein expression levels and the basic depression characteristics in the MDD group. Finally we also investigated the effects of cigarette smoking, concomitant hyperlipidemia (including complicated atherosclerosis), and current statin treatment on the *VEGFA* mRNA and protein expression levels in the MDD group.

METHODS

Subjects

This study analyzed 38 inpatients currently diagnosed with major depressive disorder (MDD) and 38 healthy control individuals. All of the patients and control subjects were native, unrelated inhabitants of central Poland.

The study group (MDD group) consisted of 38 Caucasian patients (18 females, 47.37%). All of the patients were between the ages of 23 and 79 years, 51.29 ± 11.55 (mean ± SD). They were consecutive, medication-free for at least 2 weeks at the beginning of the study, and had been admitted to a psychiatric ward between 2008 and 2010 for depression treatment. Treatment during the first week of hospitalization consisted of the following selective serotonin reuptake inhibitors (SSRIs): sertraline 100 mg/day for 14 patients; fluoxetine 20 mg/day for 3 patients, citalopram 20 mg/day for 21 patients. The patients were recruited for this study during the first week of hospitalization, just before or at the very beginning of

Table I

Demographic and clinical characteristics of the MDD group (*n*=38)

	Men (*n*=20, 52.63%)	Women (*n*=18, 47.37%)	All (*n*=38, 100%)	Difference Men vs. Women
Current age	51.90 ± 13.19	50.61 ± 9.74	51.29 ± 11.55	NS
Age at onset	42.15 ± 13.69	43.78 ± 10.53	42.92 ± 12.16	NS
Disease duration	9.95 ± 10.065	7.00 ± 6.32	8.55 ± 8.52	NS
HDRS(17)	17.85 ± 6.612	20.17 ± 4.88	18.95 ± 5.90 points Range 8–30 points	NS
Smoke cigarettes			21 (55.3)	
Hyperlipidemia			22 (57.89)	
Suicide attempts			11 (28.95)	
BMI (kg/m^2)	26.73 ± 4.43	27.93 ± 6.50	27.30 ± 5.46	NS
TCh (mg/dl)	199.90 ± 31.057	215.50 ± 57.48	207.29 ± 45.46	NS
HDL (mg/dl)	50.55 ± 10.63	52.44 ± 20.90	51.45 ± 16.11	NS
HDL (%)	25.20 ± 6.79	25.67 ± 9.90	25.42 ± 8.29	NS
LDL (mg/dl)	122.95 ± 34.64	129.67 ± 41.03	126.13 ± 37.43	NS
TG (mg/dl)	137.95 ± 78.50	165.06 ± 82.87	150.79 ± 80.67	NS

Data are presented as the mean ± SD. The *P* value for the Mann-Whitney *U*-test statistical analysis on Difference Male vs. Female is not significant (NS). [HDRS(17)] 17-item Hamilton Rating Scale for Depression; (BMI) body mass index; (TCh) total cholesterol; (LDL) low density lipoprotein; (HDL) high density lipoprotein; (TG) triglycerides.

SSRI treatment (first 2–3 days of treatment), and examined by a trained psychiatrist using the standardized Composite International Diagnostic Interview (CIDI) (Robins et al. 1981, Kessler et al. 2007). Each patient was diagnosed with MDD according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) diagnostic criteria (APA 1994). The exclusion criteria for the MDD group were other DSM-IV axis I disorders, DSM-IV axis II disorders, and neoplastic or chronic inflammatory disorders. The demographic and clinical data for the MDD group are summarized in Table I. A total of 11 patients (28.95%) had a positive family history of depression, and 21 patients (55.30%) were current smokers that smoked at least 10 cigarettes/day. The mean time of smoking was 13.45 years. A total of 22 patients (57.89%) were hyperlipidemic, and 9 of these patients were treated with 20 mg simvastatin/day for at least 3 months (prior and during hospitalization) (Table I). The mean BMI and serum lipid concentrations are listed in Table I. There were 13 patients (34.21%) with complicated atherosclerosis (11 patients with ischemic heart disease, 2 patients who had suffered a stroke). Even though each patient was hospitalized, we included the number of hospitalizations as a determining variable. We hypothesized that the higher number of hospitalization visits the patient had, the more severe was his MDD course. Our patients were hospitalized 2.61 ± 1.99 (mean ± SD) times during their lifetime. Two of our patients (5.3%) were diagnosed with diabetes.

The control group consisted of 38 healthy Caucasian subjects (25 females, 65.79%). They were between the ages of 26 and 53 years, 33.11 ± 9.51 (mean ± SD). They had a silent individual and family psychiatric history. The control subjects included community volunteers that, enrolled in the study following the criteria of the psychiatric CIDI (Robins et al. 1981, Kessler et al.

Table II

Differences in *VEGFA* mRNA and protein expression levels in the MDD group *versus* control group

	MDD group (*n*=38)	Control group (*n*=38)	*Z*/*P*
VEGFA mRNA expression	0.20 ± 0.08	0.14 ± 0.05	*Z*=3.29; *P*=0.001
VEGF serum level(pg/ml)	497.76 ± 82.49	436.42 ± 60.11	*Z*=3.49; *P*<0.001

Data are presented as the mean ± SD. (MDD) major depressive disorder group; (*VEGFA*) vascular endothelial growth factor gene; (VEGF) vascular endothelial growth factor; (mRNA) messenger ribonucleic acid; (*Z*) Mann-Whitney *U* test; (*P*) level of statistical significance.

2007). The exclusion criteria for the control group were DSM-IV axis I and II disorders, cardiovascular disease, and neoplastic and chronic inflammatory disorders.

Written informed consent was obtained from each participant. The study protocol was approved by the Local Bioethics Committee No. RNN/126/13/KB and No. RNN/598/08/KB.

Lipid serum level assessment

All the MDD patients had their lipid serum levels: total cholesterol (TCh), low density lipoprotein (LDL), high density lipoprotein (HDL), and triglycerides (TG) measured during the first week of hospitalization. Peripheral blood samples were drawn from the subjects in the morning between 07:00–09:00 AM after an overnight fast. Samples were centrifuged, and lipids were measured using enzymatic colorimetric method on the Cobas Integra 400 plus by Roche Diagnostics. Reference values for the local laboratory were TCh: 140–200 mg/dL; HDL ≥35 mg/dL; HDL ≥23%; LDL ≤150 mg/dL; and TG: 60–190 mg/dL. Patients with abnormal lipid serum levels (TCh >200 mg/dL or HDL <35 mg/dL or HDL <23% or LDL >150 mg/dL or TG >190 mg/dL) or who had been already treated with statins were considered patients with hyperlipidemia. Patients with ischemic heart disease or who had suffered a stroke were classified as patients with complicated atherosclerosis.

RNA extraction and reverse transcription

Peripheral blood samples were drawn from the subjects in the morning between 07:00–09:00 AM following an overnight fast.

The blood was collected in EDTA blood collection tubes, centrifuged at 500 g and the buffy coat layer was removed. This fraction contained the leukocytes and contaminating thrombocytes. Residual erythrocytes were lysed with 15 ml of EL Buffer (Qiagen, Valencia, CA) for 15 min at 4°C, and the leukocyte-rich fraction was collected by centrifugation (Feezor et al. 2004). After a second wash in EL Buffer, the cells were again collected, and the total RNA was extracted from the peripheral blood leukocytes using the RNA extraction reagent TRIZOL (Invitrogen Life Technologies) and following the standard acid-guanidinium-phenol-chloroform method. Approximately 5 µg of digested RNA were reverse transcribed at 42°C for 60 minutes in a total 20 µl reaction volume using the ImProm-II™ Reverse Transcription System kit (Promega, Madison WI, USA). The cDNA produced was then used in real-time PCR reactions.

Detection of gene expression using qRT-PCR method

Real-time PCR using TaqManTM technology was performed with a master mix prepared according to the FastStart Universal Probe Master (ROX) protocol from Roche Applied Science. Probes and primers were designed using the Universal ProbeLibrary (www.universalprobelibrary.com). In our study, we analyzed the following housekeeping genes from different functional classes and expressed at different levels: *ACTB* (Beta actin), *M2B (*Beta-2-microglobulin), *RPL13A* (Ribosomal protein L13a), and *GAPDH* (Glyceraldehyde-3- phosphate dehydrogenase).

The real-time PCR data confirmed that the *GADPH* gene was validated as an accurate normalization factor for this study. The following primer sequences and probe numbers were used: *VEGFA* (forward, 5-tgccgctgctgtctaat-3, reverse, 5-tctccgctctgagcaagg-3, probe: #1) and *GAPDH* (forward, 5-agccacatcgctcaga-

Table III

Association between *VEGFA* mRNA expression level, VEGF protein level and baseline depression characteristics of the MDD group

	VEGF serum level	*VEGFA* mRNA expression
Current age	R=−0.11; P=0.505	R=−0.13; P=0.445
Age at onset	R=−0.14; P=0.417	R=−0.13; P=0.430
Disease duration	R=−0.02; P=0.907	R=−0.5; P=0.782
Number of Hospitalization	R=−0.17; P=0.314	R=0.19; P=0.244
HDRS (17)	R=−0.20; P=0.226	R=−0.18; P=0.282
BMI	R=−0.30; P=0.068	R=−0.24; P=0.144
HDL	R=0.18; P=0.286	R=0.13; P=0.454
TG	R=−0.08; P=0.640	R=−0.07; P=0.689
LDL	R=0.05; P=0.75	R=0.02; P=0.089

(MDD) major depressive disorder group; (*VEGFA*) vascular endothelial growth factor gene; (VEGF) vascular endothelial growth factor; (mRNA) messenger ribonucleic acid; [HDRS(17)] 17-itemic Hamilton Rating Scale for Depression; (BMI) body mass index; (TCh) total cholesterol; (LDL) low density lipoprotein; (HDL) high density lipoprotein; (TG) triglycerides; (R) Spearman's rank correlation coefficient; (P) level of statistical significance.

cac-3, reverse, 5-gcccaatacgaccaaatcc-3, probe: #60), which was used as an internal control for real-time PCR.

Real-time PCR was conducted in a final total volume of 50 µl containing, 0.05 µg cDNA, 25 µl FastStart Universal Probe Master (ROX) 2×, 250 nM probe and 1 nM of each primer. The following amplification program was performed: 10 minutes at 95°C to activate FastStart Taq DNA polymerase followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C for amplification and signal analysis. The ABI Prism 7000 Sequence Detection System from Applied Biosystems was used to detect the amplified product. Each sample was analyzed in triplicate in independent reactions. Real-time PCR data were automatically calculated using the data analysis module.

The results were analyzed using the $2^{-\Delta\Delta Ct}$ method (Winer et al. 1999, Livak and Schmittgen 2001). Validation of PCR efficiency was performed using a standard curve. The *VEGFA* gene expression level presented as the amount normalized to the reference gene expression level, and was calculated as $2^{-\Delta\Delta Ct}$. A total of 3 separate RNA preparation were made for each sample and analyzed. Each sample was analyzed independently, and the results from the $2^{-\Delta\Delta Ct}$ calculation were averaged.

ΔCt sample = ct *VEGFA* gene − ct reference GADPH gene

ΔCt calibrator sample = ct calibrator *VEGFA* gene − ct reference calibrator GADPH gene

−ΔΔct for each analyzed sample = Δct sample − Δct calibrator

The calibrator sample was the average of the ct results from all of the study samples for corresponding *VEGFA* gene and GADPH gene.

R=$2^{-\Delta\Delta Ct}$ if R is 1, then the expression level of the studied gene is the same in the calibrator and study sample. If R is less than 1, then the *VEGFA* gene expression level is lower than the calibrator sample.

Determination of serum VEGF levels using Enzyme-Linked Immunosorbent Assay (ELISA)

For the quantitative analysis of circulating serum VEGF, the RayBio® Human VEGF ELISA (Enzyme-Linked Immunosorbent Assay) from RayBiotech was used. Serum was separated from the peripheral blood sample by centrifugation, and samples were stored at −80°C until VEGF analysis. The reproducibility of the results was determined using 3 independent experiments. Each assay was conducted with 3 replicates of

Table IV

Differences between *VEGFA* mRNA expression level and protein serum level in selected MDD subgroups

	VEGFA mRNA expression level	VEGF serum level
Gender (W=18, M=20)	Z=−0.86; P=0.388	Z=−0.42; P=0.672
Smoking cigarettes (n=21)	Z=1.04; P=0.297	Z=0.73; P=0.463
Hyperlipidemia (n=22)	Z=0.04; P=0.965	Z=0.62; P=0.535
Treatment with statins (n=9)	Z=−0.14; P=0.891	Z=−0.45; P=0.655
Complicated atherosclerosis (n=13)	Z=1.71; P=0.088	Z=1.20; P=0.230
Suicide (n=11)	Z=−2.01; P=0.044	Z=−2.22; P=0.026

(MDD) major depressive disorder group; (*VEGFA*) vascular endothelial growth factor gene; (mRNA) messenger ribonucleic acid; [HDRS(17)] 17-itemic Hamilton Rating Scale for Depression; (W) women; (M) men; (BMI) body mass index; (TCh) total cholesterol; (LDL) low density lipoprotein; (HDL) high density lipoprotein; (TG) triglycerides; (Z) Mann-Whitney U test; (P) level of statistical significance.

10 serum samples containing different concentrations of VEGF. Two standard curves were run on each plate. The calculated overall inter-assay coefficient of variation was 10.3%. The protocol and calculation of the results were performed according to the manufacturer's recommendations. Standards and samples were added to the wells with immobilized antibody specific for human VEGF and incubated. After the incubation and washes biotinylated antihuman VEGF antibody was added. After washing to remove any unbound substance, biotinylated antibody, horseradish peroxidase-conjugated streptavidin, was added to the wells. The wells were again washed, and a tetramethylbenzidine (TMB) substrate solution was added to the wells. The color developed in proportion to the amount of bound VEGF. The color developing reaction was stopped (Stop Solution), and the intensity of the color was measured using the Thermo Labsystems Multiskan Ascent 354 from Lab Recyclers at 450 nm. The serum VEGF concentration was presented as pg/ml.

Statistical analysis

All data analyses were performed in Statistica (version 10.0). The results are presented as percentages (%) or means with standard deviations (± SD). The types of measurements were selected after analyzing of the variables tested, which showed that there was no normal distribution. P-values less than 0.05 were considered significant. We used the Mann-Whitney U-test to: (1) determine differences in current age, age at onset, disease duration, HDRS (17) rating, body mass index (BMI) and serum lipids indices between the MDD male and female patients; (2) determine differences in the *VEGFA* expression level between the MDD and control group; (3) assess associations between the *VEGFA* expression level and gender, cigarette smoking, hyperlipidemia, complicated atherosclerosis, treatment with statins and suicide attempts in the MMD group. To evaluate correlations between the *VEGFA* expression level and current age, age at onset, disease duration, number of hospitalization, HDRS (17) rating, BMI and serum lipids indices, Spearman's rank correlation coefficients were estimated.

RESULTS

Individuals in the MDD and control group were not age and sex matched. There was a significant difference in age (Z=5.57, P=0.000). The obtained results showed that patients diagnosed with MDD have significantly higher *VEGFA* mRNA and protein expression levels than the controls (P=0.001; P<0.001, respectively) (Table II).

When comparing the men and women, we found no differences in the baseline depression characteristics in the MDD group [current age, age at onset, disease duration, HDRS (17) rating], BMI or lipid serum concentrations (Table I). We found no significant associa-

tion between the *VEGFA* expression level and the baseline depression characteristics, BMI or lipid serum concentrations (Table III).

The patients who attempted suicide had significantly lower *VEGFA* mRNA and protein expression levels (Z=−2.01, P=0.044; Z=−2.22, P=0.026, respectively) (Table IV). We found no significant difference (P>0.005) in the *VEGFA* mRNA and protein expression levels in the rest of the selected MDD patients subgroups (Table IV).

DISCUSSION

In the presented study, the *VEGFA* mRNA and protein expression levels were significantly higher in the MDD patients (Table II). These results are consistent with the results from previous studies. Iga and coauthors (2007) reported a higher *VEGFA* mRNA expression in 32 MDD patients. Kahl and others (2009) examined 12 women with MDD and borderline personality disorder and found that they also had a higher *VEGFA* mRNA expression level compared with 12 healthy women. Lee and Kim (2012) showed that there was a higher VEGF plasma level in 35 MDD patients. Additionally, we also found that the higher *VEGFA* expression level in our MDD group is not affected by cigarette smoking, hyperlipidemia (including complicated atherosclerosis) or statin treatment (Table IV). The MDD and control groups evaluated in this study were not matched for sex and age. The exclusion criteria for the control group included neoplastic and chronic inflammatory disorders and cardiovascular disease, which resulted in a control group that was younger than the MDD group. Including individuals with these disorders would have introduced a factor into our analysis that would have affected our results (Jin et al. 2000, Sun et al. 2003, Chancharoenthana et al. 2013, Gacche and Meschram 2013, Yamamoto 2013, Zhang et al. 2013). In the MDD group, there was no significant association between the *VEGFA* mRNA and protein expression levels and age, and these expression levels did not differ between the men and women (Tables III and IV). Similarly, there was no significant association between the *VEGFA* mRNA expression level and age in the control group, (R=0.09, P=0.606) and the expression level did not differ between the control men and women (Z=1.31, P=0.191). Furthermore, there was also no association between the protein level and age (R=0.01, P=0.936), and the expression level did not differ between the control men and women (Z=0.85, P=0.397) (data not shown). We found no correlation between the HDRS-17 score and *VEGFA* expression in the MDD group. It would be interesting to evaluate this correlation in healthy controls for subclinical symptoms of depression, but this type of evaluation was not the aim of our current study.

Antidepressant treatments for our MDD patients consisted of SSRIs. Blood samples for the expression study were drawn just before or at the very beginning of treatment. Therefore, the results were not affected by the antidepressant treatment. In the study by Iga and others (2007) evaluating 32 MDD patients, the *VEGFA* mRNA levels in the peripheral leukocytes from drug-naive subjects were significantly higher than the level from the controls. Moreover, the expression levels decreased after an 8-week antidepressant treatment, and there was a significant correlation between the magnitude of decrease and signs of clinical improvement (Iga et al. 2007). Ventriglia and coworkers (2009) did not observe any change in the serum VEGF level in 25 MDD patients after 8 and 12-week escitalopram treatments compared with the VEGF level during drug-free interval. This study, however, did not include controls. Therefore, there was no data comparing the VEGF levels in an MDD and control group before antidepressant treatment.

We found no significant difference in the *VEGFA* expression level in hyperlipidemic, complicated atherosclerosis and statins-treated MDD subgroups. Studies evaluating the relationship between total cholesterol and depression report conflicting results. A majority of the findings indicate that there is a connection between low cholesterol and major depression and that the total cholesterol level increases at the time of recovery. Some studies suggest that there is a correlation between low TCh and suicide attempts, which leads to the conclusion that statin treatments should not be used. More recent studies, however have not found a higher suicidal risk among subjects treated with statins (Blumberg et al. 2008). Blann and colleagues (2001) found higher VEGF-serum concentrations in patients with hyperlipidemia (with and without atherosclerosis) compared with healthy controls and suggested that this level can be reduced with successful lipid-lowering treatment. Giurgea and coauthors (2006) found that six

weeks of treatments with 20–40 mg of simvastatin results in a significant decrease in the VEGF serum levels in hypercholesterolemic patients.

We found no significant difference in the *VEGFA* expression level between the MDD smokers and nonsmokers. Schmidt-Lucke and coworkers (2005) and Belgore and others (2000) showed that there was no difference in the VEGF plasma concentrations between clinically healthy smokers and nonsmokers. In the study by Schmidt-Lucke and colleagues (2005), quitting smoking resulted in a significant weight gain and an elevated HDL plasma concentration.

Additionally, two of our patients were diagnosed with diabetes. One was a female, classified as a hyperlipidemic patient in this study and, taking simvastatin. The other patient was a hyperlipidemic male with complicated atherosclerosis (ischemic heart disease) and, also taking simvastatin. It has been shown that diabetics have a higher VEGF concentration, but this concentration increase was observed in the aqueous humor (Funatsu et al. 2002). Our 2 diabetic patients had *VEGFA* mRNA and protein expression levels that were lower than the mean MDD group levels. Therefore, we believe that, the inclusion of two diabetic patients in the MDD group did not affect the results in this study.

Our low sample number was a major limiting factor in this study, and the results obtained from this study should be confirmed in future studies using larger sample size.

CONCLUSION

VEGF is one factor that is expressed at higher levels in depressed patients compared with healthy subjects. This finding suggests that VEGF lays potentially significant role in the pathogenesis of MDD. Furthermore, the significantly higher *VEGFA* expression level in patients with depression appears to be unaffected by cigarette smoking, hyperlipidemia (including complicated atherosclerosis), treatment with statins or gender. These findings, however, require further investigation using a larger sample size.

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