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Peripheral Vascular Endothelial Growth Factor Changes after Transcranial Magnetic Stimulation in Treatment Resistant Depression

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Abstract

Objectives: To determine if Vascular Endothelial Growth Factor (VEGF) changes with transcranial magnetic stimulation (TMS) in treatment resistant major depressive disorder (MDD).

Methods: Serum from a naturalistic population of 15 patients with MDD was collected at baseline and after standard TMS treatment. VEGF concentration was determined via Enzyme-linked Immunosorbent Assay. Inventory of Depressive Symptomatology Self Report and Patient Health Questionnaire were used as a measure of depression symptom severity, clinical response and remission. Mann-Whiney U and Kendall's Tau Correlation were used for continuous variables.

Results: VEGF increased from pre- to post-TMS (+30.3%) in remitters whereas VEGF decreased in nonremitters (−9.87%) ($p < 0.05$). This same pattern was observed when comparing mean %change in VEGF between responders (+14.7%) and non-responders (−14.9%) ($p = 0.054$). Correlation was present between change in VEGF concentration (baseline to post) and change in IDS-SR at Tx30 ($r = -.371$, $p < 0.054$), reflecting greater increases in VEGF linked to greater improvement in depressive symptoms following the standard 6-week course of TMS.

Conclusions: Patients with a successful treatment with TMS had significantly greater increase in VEGF from baseline to after treatment compared to non-responders/non-remitters and a larger increase in VEGF was associated with greater improvement in depressive symptoms after TMS. This is the first report examining VEGF levels in depressed patients receiving TMS. This study provides correlative data supporting further investigation into VEGF's role as an important

mediator in the processes underpinning TMS' antidepressant effects and as a potential biomarker of clinical outcomes.

Keywords

Transcranial Magnetic Stimulation; Treatment Resistant Major Depressive Disorder; Vascular Endothelial Growth Factor; Biomarker; Naturalistic Study Sample

1. INTRODUCTION

Major depressive disorder (MDD) is a severely debilitating disorder affecting more than 17.3 million adults in the US alone (National Survey on Drug Use and Health, 2017) and is the leading cause of disability worldwide [1]. While antidepressant medications and nonspecific placebo effects or psychotherapy clearly benefit some depressed patients [2], a substantial number do not respond to multiple adequate trials of pharmacotherapy and have treatment resistant MDD (TRD), resulting in prolonged periods of suffering and chronic poor quality of life.

Like most neuropsychiatric disorders, the pathophysiological etiology for depression is lacking, although several have been hypothesized such as the neurotrophin theory. This theory initially focused on Brain Derived Neurotrophic Factors (BDNF) and the role of neurogenesis as a key factor of depression [3], but it has since expanded to include other neurotrophins such as the Vascular Endothelial Growth Factor (VEGF) [4,5].

Vascular Endothelial Growth Factor (VEGF) is a widely expressed endothelial mitogen in the brain involved in a myriad of functions including angiogenesis, neurogenesis, synaptic plasticity, normal brain development and maturation, and vascular permeability modulation contributing to the blood brain barrier [6,7]. While there are some heterogeneity in the literature, cumulative evidence highlighted by two recent meta-analyses [4,5] suggests that peripheral VEGF concentrations are elevated in patients with depression. Several findings have also suggested that VEGF involvement may play a key role in the effectiveness of antidepressant pharmacotherapy, though there are a limited number of human studies [8]. Interestingly, there is some evidence that VEGF may play a role as either a treatment response prediction biomarker or as a downstream target of electroconvulsive therapy (ECT) [9,10], a neuromodulatory treatment for TRD.

Transcranial Magnetic Stimulation (TMS) is a FDA approved non-invasive neuromodulatory treatment for TRD that involves delivery of fluctuating magnetic fields to induce electrical currents in targeted regions of human cortex [11,12]. Initially used as a research tool to transiently manipulate neuronal excitability, serial applications of TMS were subsequently found to have long-term therapeutic value. And although not all studies have shown positive results [13], large randomized controlled trials and naturalistic studies have shown TMS to be effective against disorders such as TRD [11,12]. However, the mechanism of action underlying the beneficial effects of TMS is still being elucidated. There have been several promising individual studies, there is no biomarker in clinical use to guide patient selection or inform optimal stimulation parameters [14–16].

Given the likelihood of VEGF playing an essential role in MDD pathophysiology and treatment pathway, it holds promise as a viable target of investigation in order to further understand the mechanism of TMS, leading to improved treatment effectiveness for patients in a more personalized medicine approach. This study aims to provide data for the investigation of peripheral VEGF levels in TRD patients receiving a standard course of TMS therapy.

2. METHODS

2.1 Study Design/Participants

This was a single center, prospective study in a naturalistically treated sample of patients who were scheduled to initiate TMS treatment for TRD in an outpatient clinic. Nineteen consecutively admitted patients aged 18 or older between 7/15/2019 and 12/4/2019 were approached and invited to participate prior to initiation of the first TMS session. All met DSM-5 criteria for primary MDD and met criteria for insurance coverage of TMS for TRD, which typically is defined by a documented history of nonresponse to multiple pharmacotherapy trials and a course of psychotherapy in the current depressive episode. There were no additional exclusion criteria beyond those applied for routine care, i.e., patients with bipolar disorder, psychotic depression or psychotic disorders are not eligible. Comorbid neurological disorders, such as seizure disorder, cerebrovascular disease, and intracranial pathology, also render depressed patients ineligible for TMS. Information regarding the study was introduced to patients during the TMS clinical service intake process by the consulting physician. Participation was voluntary and written informed consent was obtained on the Institutional Review Board-approved consent form after all procedures and risks were explained. All patients were on stable (ineffective) medication regimens at time of referral to the clinic and were directed not to change medications during the course of TMS, per standard clinical practice [17].

The final sample size was determined by the maximum number of specimens that could be tested in the assay kit we selected, i.e., 15 patients each with blood from two time points. Two patients invited to the study underwent TMS but declined to participate in the phlebotomy procedure. Of the 19 who consented, specimens could not be obtained from 2 due to limited venous access. Two participants who contributed pre-treatment blood terminated their TMS course early and did not provide post-treatment specimens for analysis.

Of the 15 patients who provided blood at both timepoints, 6 (40%) were male, and the average age was 50.73 ± 15.86 years. Five (33.3%) had received ECT in the past, and 10 (66.7%) had been hospitalized at least once in the past for psychiatric reasons. The average number of total TMS treatments was 37.7 ± 4.4 ; 10 participants received 36 treatments and three patients received a total of 46 because 10-session acute course extensions were approved by their insurance companies.

2.2 TMS Treatment and Assessments

All patients underwent a FDA approved standard treatment protocol with a figure-8 TMS coil placed over the left dorsolateral prefrontal cortex. Stimulation was administered at 10 Hz, with intensity of 120% relative to motor threshold, in 4 seconds trains, for a total of 3000 pulses per session [11,17]. Treatments were scheduled for 5 sessions/week, typically for 6 weeks, followed by three weeks of tapering frequency[11,17]. Depression severity was measured via the Inventory of Depressive Symptomatology-Self Report (IDS-SR), which has a high reliability as shown by a cronbach's alpha of 0.94 [18] and the Patient Health Questionnaire-9 (PHQ-9) [19] serially including at baseline (prior to first TMS), immediately after the 30th TMS session, and again after the final session in the taper phase. Clinical response was defined on both scales by a 50% reduction in score from baseline to post-treatment. Remission was defined by post-treatment IDS-SR score ≤ 14 [18]. On the PHQ-9 scale, remission was defined as post-treatment score ≤ 5 [19].

2.3 Serum Collection

Venous blood samples were drawn in the TMS Clinic at baseline (within a week prior to receiving first session of TMS) and within 15 minutes after TMS Session #30 (Tx30), which represents the end of a standard acute phase. Blood was allowed to clot at room temperature for 30 minutes, then centrifuged at 3000g for 15 minutes at 4°C and stored at -80°C until assay.

2.4 Enzyme Linked Immunosorbent Assay (ELISA)

Serum concentration of VEGF was measured using a commercially available sandwich ELISA kit according to the manufacturer's instruction (DVE00; R&D Systems, Minneapolis, MN, USA). The coefficient of variance for serum samples as listed on the manufacturer's data sheet is 0.045–0.067. All analyses, including the standards, were performed in duplicates and the average intra-assay coefficient of variance for the samples was 0.049. All concentrations were above the manufacturer's reported minimum detectable dose of 9 pg/ml.

2.5 Statistical Analysis

Descriptive statistics were used for characterizing the sample and their clinical outcomes at Tx30 and final endpoints; TMS treatment outcomes were not the focus of this investigation. The planned statistical analyses were intended to evaluate (1) whether VEGF changed following a course of TMS therapy; using Tx30 outcome measures because they align temporally with the collection of blood for VEGF (2) whether changes in VEGF concentration were related to symptom change again using baseline and Tx30 data; (3) whether pre-treatment VEGF concentrations were predictive of treatment outcomes; for this final set of tests, we were interested not only in prediction of Tx30 outcomes, but also prediction of the final assessment outcomes. Paired t-tests were used to compare mean VEGF concentrations from pre- and Tx30 TMS time points. Given the small sample size in the groups, Mann Whitney U test was performed to compare changes in mean VEGF concentrations between groups (remitters vs non-remitters and responders vs non-responders) and Kendall's Tau Correlation was performed to evaluate associations between

VEGF concentration and depression severity. P values are reported with statistical significance defined as $p < 0.05$ and not adjusted for multiple comparisons. Effect sizes (Cohen's d) are reported where appropriate. All data analysis was performed using SPSS statistical software (SPSS Inc. Chicago, IL).

3. RESULTS

3.1 Clinical Outcomes

The average baseline IDS-SR score was 47.73 ± 12.53 and the average baseline PHQ-9 was 20.07 ± 5.27 . Overall, at Tx30, participants experienced $52.4 \pm 24.9\%$ improvement on the IDS-SR and $54.8 \pm 37.9\%$ improvement on the PHQ-9. At end of the taper phase, overall improvement in IDS-SR was $64.4 \pm 30.1\%$ and PHQ9 was $65.7 \pm 40.4\%$. On the IDS-SR after 30 treatment sessions, eight (53%) reached response status and four (27%) had reached remission. However, after the final taper phase session, 12 (80%) reached the response criterion and nine (60%) reached the remission threshold after the final session in their taper phase. Based on PHQ-9 scores, 12 (80%) were responders and five (33%) were remitters after 30 treatments, whereas 12 (80%) achieved response and 10 (66%) reached remission by the end of their taper phase.

3.2. VEGF

3.2.1 Change in VEGF Over time: Treatment #30 Outcomes—Overall, VEGF mean concentration did not differ from baseline (258.09 ± 135.35 pg/ml) to Tx30 (254.02 ± 122.28 pg/ml). However, change in VEGF from baseline to Tx30 appeared to be related to antidepressant effect of TMS. Amongst patients who achieved remission on the IDS-SR at Tx30, mean VEGF increased from baseline to Tx30 by $30.3 \pm 37.7\%$, whereas in nonremitters VEGF decreased ($-9.87 \pm 19.1\%$; $p < 0.05$; Cohen's $d = 1.34$) (Fig. 1). This same pattern was observed with IDS-SR outcomes at Tx30 when comparing mean %change in VEGF between responders ($+14.7 \pm 32.1\%$) and non-responders ($-14.9 \pm 18.9\%$; $p < 0.05$; Cohen's $d = 1.12$; Fig. 1). With regard to outcomes defined by the PHQ-9 scale, remitters also had significantly higher increases in VEGF over time compared to non-remitters ($23.4 \pm 36.1\%$ vs $-10.4 \pm 20.0\%$; $p < 0.05$; Cohen's $d = 1.16$; Fig. 2). However, a significant difference was not observed on PHQ-9 between responders vs non-responders ($p = .665$). A statistically significant positive correlation was present between % change in VEGF concentration (baseline to Tx30) and % change in IDS-SR score at Tx30 ($r = -.371$, $p < 0.054$), reflecting greater increases in VEGF linked to greater improvement in depressive symptoms following the standard 6-week course of TMS.

3.2.2 Prediction of TMS Outcomes from Baseline VEGF—Baseline serum VEGF concentrations were statistically higher for IDS-SR remitters at the end of the taper phase, compared to non-remitters (308.44 ± 146.42 vs 182.57 ± 74.75 , $p < 0.05$). Although not statistically significant, patient subgroups defined by positive response to TMS (e.g., IDS-SR responders, PHQ-9 remitters and PHQ-9 responders) all had numerically higher baseline serum VEGF concentration compared to their counterparts as well (Fig. 3), providing a preliminary signal for the potential utility of VEGF as a pre-treatment biomarker for

response prediction. However, baseline serum VEGF concentration did not have a statistically significant correlation with %change in IDS-SR or PHQ-9 at the final TMS.

4. DISCUSSION

This is the first study to examine peripheral VEGF in patients with depression receiving TMS. The results provide evidence that successful TMS treatment was associated with change in VEGF concentration. Specifically, remitters and responders to 30 TMS sessions had increases in VEGF, whereas those who did not experience similar symptomatic improvement had an overall VEGF decrease. Patients with the greatest VEGF increases tended to have the lowest level of residual symptoms after their course of TMS. While the sample size was small for this study, our findings suggest that (1) VEGF concentrations change over time in association with resolution of depressive symptoms in the context of a standard course of TMS therapy (2) higher levels of VEGF appear to be associated with lower levels of depressive symptom severity, and (3) among depressed patients referred for TMS therapy, those who fare well with the treatment could be predicted by VEGF concentrations at baseline. Our findings were more robust in outcomes defined by one of the two self-report depression symptom measures we use: the IDS-SR scale. Less signal was detected when treatment outcomes were defined by the PHQ-9 scale. Designed as a screening tool for primary care settings, the PHQ-9 is a 9-item survey which does not assess anxiety/tension or numerous other of 28 common MDD symptoms that are captured by the IDS-SR (e.g., diurnal variation, irritability, quality of mood, aches and pains, gastrointestinal symptoms, panic/phobic symptoms, interpersonal sensitivity, and other types of somatic symptoms).

Similar to the present findings, serum levels of VEGF were shown to increase after ECT in patients with TRD, and the authors also found a significant correlation between VEGF increase and reduction in depression severity [10]. A cohort study following an inpatient sample of TRD patients for 6 months also showed a decrease in serum VEGF in non-responders to a mixture of treatments including pharmacological, psychotherapy, and ECT [20]. There have been mixed reports regarding VEGF changes after antidepressant pharmacotherapy, with studies of escitalopram, quetiapine [21] or individualized pharmacotherapy [22,23], showing no change over time. However, response to duloxetine therapy was associated with an increase in VEGF levels, and nonresponse with a decrease [24]. Whether the increase in VEGF we observed is related to TMS or represents a nonspecific response to successful antidepressant treatment in depressed patients is a question to be answered with further studies.

Similar to our results, lower VEGF levels in depressed patients prior to initiation of therapy has been shown to predict inferior antidepressant treatment outcomes in several studies including ECT [9,25] and individualized pharmacotherapy in an inpatient setting for TRD [26], and escitalopram or quetiapine for non-resistant MDD [21]. One study which looked at a small sample that included both MDD and Bipolar depressed patients showed a non-statistically significant trend toward higher baseline plasma VEGF concentrations in non-responders compared to responders [22].

Although an effect of TMS therapy on VEGF in humans has not previously been demonstrated, TMS has been shown to increase VEGF brain levels in rat models of vascular dementia via bilateral carotid artery occlusion [27] and in Parkinson's models using striatal injection of 6-hydroxydopamine [28]. In the present dataset, there was a differential change in VEGF concentration associated with TMS such that increases in VEGF characterized those with clinical improvement. An increase of VEGF in association with successful antidepressant treatment may be related to biological processes relevant to depression that are not shared with ischemic dementia or Parkinson's Disease.

Although there is considerable methodological heterogeneity amongst published studies, a recent meta-analysis has shown that overall, MDD patients likely have higher peripheral VEGF levels compared to healthy controls [4,5]. One hypothesis regarding this observed pattern may be a compensatory mechanism in which VEGF is overexpressed in the stressed brain to exert its neuroprotective roles, including its actions in angiogenesis, neurogenesis, gliogenesis, neuroplasticity, and neural stem cell proliferation in the hippocampus. Furthermore, some have hypothesized that increased VEGF could aid in increasing antidepressant delivery to the brain by downregulating multi-drug resistance proteins at the blood brain barrier [9,20]. Thus one model to differentiate TRD from non-TRD MDD at a physiological level could be that in TRD, the compensatory mechanism to increase VEGF under stressful conditions such as MDD is altered. This may explain why some studies have shown no difference in VEGF between depressed patients and healthy controls.

CONCLUSION

Given the promising result of this study, future studies will be conducted with an expanded sample size to allow for increased power to test the usefulness of baseline VEGF as a clinical biomarker of TMS treatment response. Although a naturalistic approach was chosen for this pilot study, utilization of randomization and a sham-controlled design is needed to determine whether there is a placebo effect on VEGF concentrations. Future studies may also utilize additional functional or psychometric assessment tools to correlate VEGF changes with specific depressive symptoms or symptom clusters to delineate the functional meaning of changes in VEGF levels. It will also be important to understand whether VEGF increases are durable and associated with remission from depression over the long-term. Importantly, future studies will determine whether the changes we observed with TMS are related to brain stimulation treatment or are nonspecific and simply represent correlates of any successful antidepressant treatment modality.

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Figure 1 –. Change in VEGF Over Time: Effect of IDS-SR Outcome Groups.

(A) A statistically significant difference in % change of VEGF was present between remitters (n=4) and non-remitters (n=11) ($U=6.000$, $p=0.040$), as can be seen by (C) an increase in mean concentration of VEGF from baseline to tx30 in remitters, and a decrease in mean concentration in non-remitters. (B, D) A similar pattern was seen with responders (n=8) vs non-responders (n=7) ($U=11.000$, $p=0.054$) as well.

* = $p<0.05$, VEGF = Vascular Endothelial Growth Factor, tx30 = after treatment #30, IDSSR = The Inventory of Depressive Symptoms-Self Report

Figure 2 –. Change in VEGF Over Time: Effect of PHQ-9 Outcome Groups.

(A) A statistically significant difference in % change of VEGF was present between remitters (n=5) and non-remitters (n=10) ($U=8.000$, $p=0.040$), as can be seen by (C) an increase in mean concentration of VEGF from baseline to tx30 in remitters, and a decrease in mean concentration in non-remitters. (B, D) A similar pattern was seen with responders (n=12) vs non-responders (n=3) as well.

* = $p<0.05$, VEGF = Vascular Endothelial Growth Factor, tx30 = after treatment #30, PHQ9 = Patient Health Questionnaire-9.

Figure 3- Baseline VEGF Concentration In Outcome Groups Defined by Scores after Final Treatment

Baseline VEGF concentration was compared between remitters/responders and nonremitters/nonresponders according to IDS-SR and PHQ-9 after the full treatment course. (A) IDS-SR remitters had a significantly higher baseline VEGF concentration compared to nonremitters (308.44 ± 146.42 vs 182.57 ± 74.75 , $p < 0.05$). (C) Although not statistically significant a similar pattern of a higher baseline VEGF was observed in those who met remission criteria via the PHQ-9 compared to nonremitters (285.56 ± 156.54 vs 203.17 ± 57.65 , $p > 0.1$). (B)(D) Similarly, although not statistically significant and the differences were not as prominent, responders also had a high baseline VEGF than nonresponders.