Major Depressive Disorder (MDD): Potential Predictors of Vulnerability and Treatment Response

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Major Depressive Disorder (MDD) is a common and disabling brain disorder affecting millions world-wide. Despite decades of research and advances in drug development, response to antidepressant treatment remains highly variable and less than optimal. Further investigation of the role of biomarkers, genes, pharmacokinetics, pharmacodynamics and neuroimaging are needed to elucidate the underlying pathophysiology of depression. Among the neurobiological hypotheses, the role of pro-inflammatory cytokines, neurotrophic factors and deficiency of monoamines are among the most supported. The research that comprises this dissertation aims to identify potential predictors of depression vulnerability and antidepressant treatment response.

The first study investigated the relationship between the BDNF Val66Met polymorphism, serum BDNF levels, and the development of depressive symptoms in a clinical study of 149 patients receiving interferon-alpha (INF-α) therapy for treatment of Chronic Hepatitis C. Here we report an association between lower baseline BDNF levels and higher depressive symptoms during IFN-α treatment. The Met allele was associated with lower BDNF levels and higher suicidal ideation, sadness and worthlessness. In addition, IFN-α therapy further decreased BDNF serum levels. Collectively, these findings support the hypothesis that BDNF improves resiliency against developing a subset of cytokine-associated depressive symptoms.

The second study in patients with MDD investigated variability in venlafaxine dose/drug concentrations and treatment response including clinical outcomes and alterations in brain functional connectivity. We observed a correlation between venlafaxine dose and drug concentration at late study time points; lower BMI and age over 65 years was associated with higher drug concentration. Higher concentration at week 1 was associated with low MADRS trajectory and there was a positive correlation between drug concentration and change in MADRS scores at week 12. Path analysis revealed indirect effect of dose on clinical outcomes which was mediated through drug concentration. These findings suggest that the efficacy and safety of venlafaxine treatment of patients with MDD may be optimized through dose titration based on therapeutic drug monitoring. Moreover, patient factors such as age and BMI should be taking into account during venlafaxine dose adjustment in the treatment of MDD.

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Preface

I am dedicating this dissertation to my wonderful parents, Ahmed Albusaysi and Hawa Adam, who encouraged me to pursue my graduate degree abroad. Their unconditional love, endless support, and continuous encouragement made this work possible. I am very grateful to have a loving and supportive family, and I feel it is my privilege to belong to them. Words cannot express my sincere gratitude to my parents. My father valued education and he encouraged me to choose pharmacy as my career path. He has been very supportive since the beginning of my journey. He always reminds me to push through the struggles and see the greater value that will come later. My mother has sacrificed her time and effort to make sure that I do not lose focus on my goals. I also want to thank my siblings: Mona, Fatimah, Zainab, Bayan, Joumanna, Shimma, Mohammed and AbdulRahman for being very loving and understanding although the geographic distance made it hard to stay in touch. Specially, I would like to thank my twin sister, Mona, for her friendship and support during my PhD journey.

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List of Abbreviations

5-HT	Serotonin
5-HTTLPR	Serotonin Transporter
ACC	Anterior Cingulate Cortex
BDI-II	Beck Depression Inventory
BDNF	Brain-Derived Neurotrophic Factor
BMI	Body Mass Index
BOLD	Blood-Oxygen-Level Dependent
CHC	Chronic Hepatitis C
CIRS-G	Cumulative Illness Rating Scale-Geriatric
CNS	Central Nervous System
CYP	Cytochrome P450
DLPFC	Dorsolateral Prefrontal Cortex
DMN	Default Mode Network
DSM-5	Diagnostic and Statistical Manual of Mental Disorders- 5th Edition
ECN	Executive Control Network
ELISA	Enzyme Linked Immuno-Sorbent Assay
fMRI	Functional Magnetic Resonance Imaging
GI	Gastro-Intestinal
GWAS	Genome-Wide Association Studies
HCV	Hepatitis C virus

HPLC-MS/MS	High-Performance Liquid Chromatography with tandem Mass Spectrometry
IFN-α	Interferon-alpha
IIV	Inter-Individual Variability
IL	Interleukin
ILP	Inferior Parietal Lobule
LLQ	Lower Limit of Quantification
LLQ	Lower Limit of Quantitation
lMTG	left Middle Temporal Gyrus
MADRS	Montgomery-Asberg Depression Rating Scale
MAOIs	Monoamine Oxidase Inhibitors
MAP	Mitogen Activated Protein
MAP	Mitogen Activated Protein
MDD	Major Depressive Disorder
Met	Methionine
MHC	Major Histocompatibility Complex
MMSE	Mini-Mental State Examination
mPFC	medial Prefrontal Cortex
NE	Norepinephrine
NF-kappaB	Nuclear Factor Kappa-light-chain-enhancer of activated B cells
NONMEM	Non-Linear Mixed Effect Model
ODV	O-desmethylvenlafaxine
PCC	Posterior Cingulate Cortex

PD	Pharmacodynamics
PEG-IFN-α	Pegylated IFN-α
PK	Pharmacokinetics
rIFG	right Inferior Frontal Gyrus
rMTG	right Middle Temporal Gyrus
ROI	Region Of Interest
rPCG	right Precentral Gyrus
rSMG	right Gupramarginal Gyrus
SCID-IV	Structured Clinical Interview for DSM-IV Axis I Disorders
SEM	Structural Equation Model
SLC6A4	Serotonin Transporter Gene
SNP	Single Nucleotide Polymorphism
SNRIs	Serotonin-Noradrenaline Reuptake Inhibitors
TCAs	Tricyclic Antidepressants
TDM	Therapeutic Drug Monitoring
TNF-α	Tumor Necrosis Factor-α
TrkB	Tyrosine Receptor Kinase-B
Val	Valine
WHO	World Health Organization

1.0 BACKGROUND AND INTRODUCTION

1.1 Major Depressive Disorder (MDD)

Major Depressive Disorder (MDD) is one of the most common mental disorders affecting more than 300 million people (4.4% of the global population) and will become the leading cause of disability worldwide by the year 2030.[1] According to a recent report by the World Health Organization (WHO), the number of individuals suffering from depression has increased by 18.4% between 2005 and 2015. The estimated lifetime prevalence of MDD in the United State is 16.6%.[2]

MDD is an affective disorder characterized by the presence of clinical symptoms including depressed mood and generalized loss of interest or pleasure. According to the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5)[3], the diagnosis of MDD requires the presence of at least five of the nine symptoms listed in Table 1. The core symptoms of depressed mood continuously for 2 weeks or loss of interest in normal daily activities must be one of the five required symptoms.[4] In addition to the diagnostic evaluation, the severity of MDD symptoms can be assessed objectively using the standardized scales such as Montgomery Åsberg Depression Rating Scale (MADRS) and Beck Depression Inventory (BDI-II). Recent studies comparing

MADRS and BDI-II scales have demonstrated that both scales have good comparability and reliability.[5]

Depression is a chronic and disabling condition that can reduce the quality of life and increase the cost to society. The annual economic burden of depression is estimated at $83.1 billion in 2000 in the United States with two thirds of this cost attributed to impaired productivity and absence from work.[6] In addition, depression can increase risk for developing multiple medical illness including diabetes, myocardial infarction and drug addiction as well as lead to increase mortality.[7-9] Despite certain advances in the pharmacological treatment of depression over the past five decades, it is estimated that only about half of patients respond to the initial antidepressant and even fewer, roughly one third, will achieve a complete response.[10] Those patients who fail to remit are at a significantly increased risk of relapse.[10] Thus, inadequate response to currently available antidepressants warrants the research and development of novel treatment modalities in addition to optimization of existing pharmacotherapies.

Table 1: DSM-5 Criteria for Major Depressive Disorder

Core symptoms (≥1 required for diagnosis)	Depressed mood most of the day
	Decreased interest or pleasure in almost all activities for most of the day
Additional symptoms	Clinically significant weight loss or increase or decrease in appetite
	Excessive sleep or not enough sleep
	Observable psychomotor agitation or retardation
	Fatigue or loss of energy
	Feelings of guilt or worthlessness
	Poor concentration or indecisiveness
	Recurrent thoughts of death or suicide attempt

1.2 Etiology of MDD

Several risk factors for MDD have been identified including: female gender, family history of depression, substance use disorders, chronic medical conditions, severe psychological events or stressors (such as childhood trauma or death of loved one) as well as certain medications (such as beta blockers and INF-α.[11] Indeed, numerous studies consistently show that both genetic and environmental factors contribute to the development of depression. However, despite advances in our understanding of risk factors, the heterogeneity of MDD as well as limitations of clinical symptom-based diagnostic criteria makes predicting vulnerability and variability in treatment response difficult. The mechanisms leading to MDD are complex and likely involve a combination of social, psychological and biological factors. Findings from animal models of MDD and clinical studies support multiple hypotheses of the underlying pathophysiology of MDD among which include pro-inflammatory cytokines, neurotrophic factors, and monoamines.[12] Furthermore, discoveries in genetic vulnerability and advances in neuroimaging have also helped to elucidate the neurobiological basis for depression and its treatment.

1.2.1 Inflammation

Cytokines play a critical role in the communication between the immune system and the central nervous system (CNS).[13] There is a growing evidence suggest that the pro-inflammatory cytokines play an important role in the pathophysiology of depression. Recent meta-analyses studies comparing depressed and control healthy subjects have identified significant increase in 12 inflammatory proteins in depressed patients, which include interleukin (IL) 1α, 1β, 6 and tumor necrosis factor-α (TNF-α).[14] In addition, receptors for these cytokines are present through the brain

especially in brain structures highly connected with depression such as the hippocampus and hypothalamus.[14] These pro-inflammatory cytokines have shown to modulate neurotransmitter metabolism, neuroendocrine systems, synaptic plasticity and behavior.[15]

Clinically, the cytokine INF-α has been used to treat various disorders such as malignant melanoma and chronic hepatitis C virus infection. INF-α has both antiviral and immunomodulatory effects. It facilitates the recognition of the virus-infected cells by immune cells through inducing the expression of a specific group of glycoprotein called major histocompatibility complex (MHC) and adhesion molecule.[16] Furthermore, it activates immune cells such as macrophages and natural killer cells to help eradicate the virus.

Growing evidence demonstrates that chronic administration of INF-α induces symptoms associated with depression such as fatigue, difficulty sleeping, irritability, reduced appetite and low mood, or so called "sickness behavior". The mechanism of INF-α induced depression is complex and not well understood. Studies suggest that INF-α can act directly on the CNS to induce depression or indirectly via activation of the peripheral pro-inflammatory cytokines. Moreover, clinical studies demonstrate that administration of INF-α in human results in stimulation of IL-6, IL-1, TNF-α production. These proinflammatory cytokines can affect the brain neurotransmitter and thus induce depression.[12] In addition to the effect of INF-α on neurotransmitters, growing evidence suggests that inflammatory cytokines can reduce the levels of growth factors and neurotrophins ultimately leading to reduced neurogenesis and neuronal plasticity.[17] Interestingly, clinical studies reported that about 30-50% of patients taking INF-α develop depressive symptoms

similar to that of MDD. These findings suggest further research is needed to investigate the pathophysiology of cytokine-induced depression.

1.2.2 Brain-Derived Neurotrophic Factor (BDNF)

Neurotrophic factors play an important role in supporting neuronal structure and function. One of the most extensively studied trophic factors in the CNS is brain derived neurotrophic factor (BDNF). BDNF is widely expressed in the adult brain with highest levels being found in the hippocampus and cerebral cortex.[18] BDNF has been shown to bind with tyrosine receptor kinase-B (TrkB) to promote neuronal survival and differentiation. There is a growing evidence supporting the role of BDNF in many psychiatric disorders including depression. Preclinical studies demonstrated that chronic stress is associated with reduced BDNF levels in the hippocampus.[19] In addition, chronic stress has been shown to activate the hypothalamus-pituitary adrenal (HPA) axis, which result in increased glucocorticoids levels and reduced BDNF mRNA expression in multiple brain regions including hippocampus[20]. The molecular mechanism by which glucocorticoids can impact BDNF levels is not well understood. However, previous studies have reported that administration of glucocorticoid agonist, dexamethasone, results in downregulation of BDNF mRNA expression by ~30% and this effect was abolished following administration of glucocorticoid antagonist.[21] In addition, previous studies have shown that glucocorticoid receptors directly downregulate BDNF expression through binding to a specific DNA region upstream of exon IV[21]. Interestingly, the chronic administration of antidepressants in animals has been shown to increase the production of hippocampal BDNF levels and the infusion of BDNF protein into the midbrain has an antidepressant-like effect in animal models of depression.[22] Based on these preclinical and clinical findings, BDNF represents an attractive target for treatment of depression.

1.2.3 Genetics

Numerous literature reviews report that genetics play a significant role in the pathogenesis of MDD. Candidate gene studies and genome-wide association studies (GWAS) have been utilized to identify candidate genes associated with MDD. For example, numerous genes involved in the serotonergic system, such as serotonin transporter gene (SLC6A4), has been shown to be more frequent in MDD patients compared to controls.[23] In addition, recent studies are investigating the association between functional Val66Met polymorphism in BDNF gene and the susceptibility for depression. This single nucleotide polymorphism (SNP) in the BDNF gene is caused by a G-to-A transition resulting in a change from valine (Val) to methionine (Met) in the coding exon at position 66 (Val66Met) (rs6265). Because this SNP is located in the pro-domain of BDNF gene that is cleaved off during processing of BDNF protein, it is expected that this SNP could potentially affect the intracellular trafficking, distribution, and secretion of BDNF, but it is unlikely to affect the activity of BDNF protein. One in vitro study demonstrated that the Val66Met variant is a functional polymorphism because it affects processing of the pro-BDNF polypeptide and its release when neurons are activated.[24] In a clinical study of geriatric patients with depression, the presence of the variant Met is associated with higher risk for depression. The presence of this genetic variance is associated with increased risk for suicide.[25] In addition, Val66Met SNP has been implicated in the structural changes of hippocampal volume found in depressed patients.[26] Despite all effort, so far there is no single candidate gene has been identified to increase the risk of developing depression. It is expected that multiple genes could contribute to depression.

1.2.4 Monoamine-Deficiency

The monoamine hypothesis of depression is one of the earliest theories to elucidate mechanism of depression. This theory is based on the association between reduced levels of the monoamine neurotransmitters including serotonin, noradrenaline, and dopamine and the risk for developing depression. Most of the marketed antidepressant medications have been developed to target the deficiency in the monoaminergic systems by inhibiting their metabolism or reuptake by presynaptic neurons.

1.3 Venlafaxine

The first novel antidepressant class with dual inhibitory mechanism is venlafaxine, known by the brand name Effexor®[27], which belongs to the pharmacological class of serotonin-noradrenaline reuptake inhibitors (SNRIs). This drug selectively blocks both norepinephrine (NE) and serotonin (5-HT) transporter and the administered dose of venlafaxine determine the targeted transporters. For example, venlafaxine selectively inhibits the uptake of 5-HT transporter at low does (37.5-150 mg/day) and inhibits both 5-HT and NE by "dual mechanism" at higher doses (150-300 mg/day).[28]

1.3.1 Venlafaxine Pharmacokinetics and Pharmacodynamics

The pharmacokinetics (PK) and pharmacodynamics (PD) of venlafaxine is described in the product label.[29] Venlafaxine is highly absorbed after oral administration (about 92% of the dose is

absorbed). However, the fraction of the dose that reach the systemic circulation is about 40 to 45% due to first-pass metabolism after oral administration. Venlafaxine is extensively metabolized in the liver by cytochrome P450 (CYP) enzymes, primarily CYP2D6 into an active metabolite O-desmethylvenlafaxine (ODV). Both venlafaxine and ODV have similar antidepressant activity.[29] Additionally, CYP2D6 has been shown to have many gene variants that could alter drug metabolism.[30] Venlafaxine and its metabolite are eliminated primarily through the urine. The incidence of drug-drug interaction is minimal since both compounds have minimal (~30%) binding to plasma protein including albumin. Venlafaxine and its metabolite do not have monoamine oxidase (MAO) inhibitory activity, thus dietary restriction is not a major concern.[29]

Venlafaxine demonstrated a faster onset of action compare to fluoxetine (SSRIs). Additionally, venlafaxine has proven to be effective in treating patients especially those with comorbid anxiety and treatment refractory or treatment resistant depression.[31] Venlafaxine has an improved safety and tolerability profile compare to tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs). Unlike TCAs, treatment with venlafaxine does not commonly lead to anticholinergic, sedative, and cardiovascular side effects possibly due to its poor binding affinity with histaminergic, muscarinic, or alpha1-adrenergic receptors.[32] The lack of the troublesome side effects commonly observed with other antidepressants makes it a good option for elderly patients who are more sensitive to side effects and have failed other therapies or who may have a comorbid anxiety disorder.

1.3.2 Venlafaxine Dosing

Effexor XR® (venlafaxine) is an extended-release capsule intended for once a day administration. The starting dose of venlafaxine is 37.5mg/day with subsequent increases in the dosage to 75 mg/day. The dosage then maybe increased to 150 mg/day after a week or two based on patient's tolerability and clinical response. If needed, further dosage increases are made gradually. The usual maximum daily dosage for venlafaxine is 225 mg, but more severely depressed patients may be treated with a maximum daily dose of 350 mg. Venlafaxine metabolism and elimination is reduced in patients with renal or hepatic impairment; therefore, dosage adjustment is recommended in these patients. However, dosage adjustment based on age, gender and CYP2D6 genetic polymorphism is not required. Additional details regarding venlafaxine dosing and administration can be found in the product label.[29]

1.4 Functional Connectivity in the Brain

Findings from previous research have shed the light into the interplay between functional connectivity in the brain and behavioral pathology. Neuroimaging technology offers an invaluable tool to identify brain regions that are commonly interconnected which we refer to as brain network. In addition, recent studies have utilized the functional magnetic resonance imaging (fMRI) to quantitively measure change in the brain connectivity in response to pharmacological treatment.

There are two mostly investigated network in MDD including the default mode network (DMN) and the executive control network (ECN). The DMN network in involved in self-

referential thoughts and rumination and incorporates various brain regions, including the anterior and posterior cingulate, the lateral parietal lobes, and the medial and lateral temporal regions.[33] Previous studies have shown that increased activity of DMN in depressed patients is associated with negative bias and increased self-referential thoughts and rumination.[34] Furthermore, the ECN incorporates the dorsolateral prefrontal cortex and inferior parietal cortex, which are highly correlated with emotional regulation The ECN is involved in executive functioning such as working memory, cognitive control, goal-oriented- behavior and decision making.[35] Previous studied on MDD have shown that depressed patients have decreased functional connectivity in the ECN.[36] Collectively, these data suggest that the ECN and DMN networks may play a role in the underlying pathophysiology of MDD.

1.5 Path Analysis

Path analysis is emerging as a popular statistical approach in analysis of complex, inter-related clinical data. Path analysis is a type of structural equation model (SEM) used for simultaneously studying direct and indirect effects on a dependent variable.[37] Using this method, path coefficients and correlation coefficients are calculated based on the empirical model structure. The path analysis helps to simultaneously quantitate the total effect, the direct effect, and indirect effect via mediation.[38] As previously described by Streiner, a direct effect refer to the effect of the independent variable (X) on the dependent variable (Y); Indirect effects refer to the product of the relationship between X and the mediator (M) and the relationship between M and Y, and total effects refer to the sum of the direct and indirect effects[39]. Direct effects tell you how a 1unit change in X will affect Y, holding all other variables constant. However, it may be that other

variables are not likely to remain constant if X changes, e.g. a change in X can produce a change in M which in turn produces a change in Y. Put another way, both the direct and indirect effects of X on Y must be considered if we want to know what effect a change in X will have on Y, i.e. we want to know the total effects (direct + indirect).[40]

Path Analysis with SEM is similar to traditional methods like correlation and regression in many ways. However, there are several advantages of path analysis when compared to regression and other statistical method.[41] First, path analysis allows testing for multiple dependent variables however, regression allows for testing a single dependent variable. Second, path analysis allows you to specify the relationship between variables a prior and test the hypothesized model, however regression is descriptive by nature, so that hypothesis-testing is difficult. [41] Third, path analysis helps to quantify the direct and indirect effects on a dependent variable simultaneously. Given these highly desirable characteristics, path analysis has become popular method in clinical research.

1.6 Gap in Current MDD Literature

Our understanding of the pathophysiology of depression is improved with advanced neuroimaging techniques, animal models, clinical and post-mortem studies. Additionally, there are over 20 safe and effective pharmacological treatment options available in the market. However, the pharmacological treatment of depression presents significant challenges as shown by the lack of response in nearly one third of patients.[10] On average, it takes at least 4 weeks to determine if a patient will response to an antidepressant.[6] This long trial and error period can increase the risk of

suicide or lead to worsening medical co-morbidity, disability and reduced quality of life. Identifying early predictors of response can help identify patients less likely to respond to a specific treatment, minimize exposure to ineffective treatments, and potentially avoid serious adverse events associated with treatment. The use available tools from genetic studies to biomarkers to neuroimaging is the first step toward personalized treatment of depression.

While clinical studies have focused on the relationship between venlafaxine dose and clinical response, few have investigated the relationship between venlafaxine drug concentration and outcomes in MDD. In the limited clinical studies that have investigated these relationships, the authors reported high variability in venlafaxine drug exposure. In general, there is a lack of understanding of the factors affecting the PK and ultimately the PD of venlafaxine in the clinic. Furthermore, even fewer studies have evaluated the impact of venlafaxine of functional connectivity of the brain or as a potential predictor of treatment response. Use of biomarker analysis, genetic testing, modeling and simulation, as well as innovative statistical methods such as path analysis will allow us to explore each of these factors as potential predictors of depression vulnerability and variability to treatment response.

In this dissertation, we focused on two research hypotheses:

1) INF-α therapy induced depression in patients with HCV is due to reduction in BDNF levels which is more likely to occur in patients with Val66Met polymorphism.
2) In depressed patients on venlafaxine treatment, venlafaxine concentration in plasma will be associated with improved clinical outcomes and altered fMRI connectivity in key brain regions.

2.0 Brain-Derived Neurotrophic Factor Serum Levels and Genotype: Association with Depression during Interferon- α Treatment

2.1 Introduction

MDD is a common and heterogeneous syndrome. When co-morbid with other chronic diseases, the adverse health effects are worse than any other combination of chronic diseases without depression.[42, 43] There is accumulating evidence that inflammatory cytokines have the capacity to induce depressive symptoms[44, 45], and several pathways have been identified by which peripheral cytokines can influence the central nervous system[46], prompting the hypothesis that many instances of depression may have increased inflammatory cytokines as a critical element in their pathoetiology.[47-50] However, not every individual who is exposed to elevated inflammatory cytokines develops MDD, indicating a role for vulnerability and resiliency factors in moderating the adverse depressogenic effects of inflammatory cytokines.[51] Iatrogenic MDD can be triggered by treatment with an exogenous inflammatory cytokine, IFN-α. This clinical situation has become a paradigmatic model for prospectively examining vulnerability/resilience to inflammatory cytokine-associated depression, as MDD develops in about 30% of non-depressed subjects within a few months of initiating IFN-α treatment.[52, 53] A number of putative vulnerability factors for subsequent IFN-MDD have been identified ranging from a "short" (S) low-expression allele in the promoter of the serotonin transporter (5-HTTLPR)[54, 55], a polymorphism in the serotonin 1A receptor[56], increased interleukin-6 (IL-6) serum levels and an IL-6 polymorphism[55, 57], pre-existing poor sleep quality[58], high neuroticism traits[59], increased hypothalamic-pituitary-adrenal axis

sensitivity[60], elevated ratio of omega-6 fatty acids to omega-3 fatty acids[61], and increased sensitivity to activating the p38 mitogen-activated protein kinase.[62]

Accumulating evidence also supports an important role for decreased BDNF activity in inflammatory cytokine-associated depression.[63-66] Inflammatory cytokines can decrease BDNF signaling[67, 68], as can lipopolysaccharide injections.[69] Therefore, in addition to IFN-α's effects on serotonin[70], dopamine[71], glutamate[72], and the hypothalamic-pituitary-adrenal axis[73], a decrease in BDNF may ultimately be the reason for the development of depression during IFN-α treatment. Related to this, social isolation decreases central BDNF and neurogenesis, an effect which is likely mediated by the inflammatory cytokine IL-1β.[74-76] IFN-α also appears to decrease cell proliferation in the hippocampus via increased IL-1β.[77] In fact, the effects of both stress and inflammation may be mediated by impairments in growth factor function.[48, 78, 79] Moreover, many antidepressant effects likely occur through activation of BDNF's receptor[80], and even the neuroprotective effect of a tricyclic antidepressant against lipopolysaccharide-induced apoptosis requires BDNF.[78] In human bipolar populations, there is an inverse relationship between inflammatory cytokines and serum BDNF. [81] Thus, it is feasible that cytokine-induced decreases in BDNF may result in the depressogenic effects of inflammatory cytokines such as IFN-α.

Consistent with this, several studies have associated low serum BDNF with MDD[66, 82-87], which subsequently normalizes with antidepressant treatment.[88, 89] Relatedly, a functional polymorphism causing a change from valine (Val) to methionine (Met) may result in diminished BDNF secretion.[90] The Val to Met variant at amino acid 66 (Val66Met) results from a G758A polymorphism (rs6265) in BDNF's 11th exon. The Met allele has been associated with lower serum BDNF[91], though this has not been consistently replicated.[92-94] The Met allele has also been

associated with increased suicide risk[95-97], various depression-related traits[98-106], and sometimes a depression diagnosis.[107-110] However, there are multiple studies in which this association with depression has not been replicated.[111-116]

There are therefore two potential non-mutually exclusive hypotheses that we examined. One is that IFN-α therapy decreases BDNF-but only in a subset of people who subsequently develop depression. That is, we examined whether decreased BDNF might mediate IFN-α's depressogenic effect. Alternatively, prior adversity could have lasting epigenetic effects on BDNF production[117] as could the Met allele, resulting in increased vulnerability to depression. Thus, the second hypothesis is that pre-existing low BDNF increases risk for developing depression. That is, we determined whether low BDNF and/or the BDNF Met allele enhances (i.e. moderates) the depressogenic effect of IFN-α.

Finally, different genetic regions have been associated with specific mood and anxiety traits in both mice[118], and humans[119, 120], which can affect genetic association study results.[51] Consistent with this, we have found that polymorphisms affecting TNF-α and IL-28b are associated with specific mood-related symptom clusters in individuals receiving IFN-α.[121, 122] Also, we have found that a serotonin reuptake promoter polymorphism (5-HTTLPR) is associated with increased Beck Depression Inventory scores during IFN-α therapy[54], but another group did not find an association of this polymorphism when using the Hospital Anxiety Depression Scale.[56] Thus, we also explored the possibility that BDNF genotype may influence specific depression symptoms.

2.2 Materials and Methods

2.2.1 Participants

209 adult subjects with chronic HCV were screened using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-IV), as previously described in an overlapping cohort of subjects [54, 57], and as approved by the University of Pittsburgh Institutional Review Board. Anyone taking antidepressants, anticonvulsants, or antipsychotics was excluded (none were taking steroids, although most took non-steroidal anti-inflammatory medications as needed for pain and fever during the course of IFN-α treatment). Those without active mood, anxiety, psychotic, or drug/alcohol abuse disorders were assessed for BDNF levels (n=156). Of these, 124 subsequently started IFN-α treatment within 6 months–comprised of weekly injections of pegylated IFN-α2 (PEG-IFN-α2a:135 μg/week or PEG-IFN-α2b: 120 or 150 μg/week) augmented with oral ribavirin. No subjects were noted to develop incident MDD during the period between the initial baseline assessment and the start of IFN-α therapy (56±55 days).

2.2.2 Depression Assessment

Depression symptoms were assessed at baseline and then monthly during IFN-α treatment (for up to 4 months) using the Beck Depression Inventory-II (BDI-II). [123] Participants who did develop MDD during the course of treatment were started on an antidepressant. Data from individuals on antidepressant medications censored from the analyses.

The BDI-II is a widely used scale as a diagnostic screen and assessment of depression severity. This is self-rating scale and has 21-items evaluating wide range of psychological and

neuro-vegetative symptoms of depression such as feeling sad, lack of interest in sex, irritability, weight loss, hopelessness and fear of being punished. Since this scale is self-report it takes less time and does not require a clinician to administer the scale.

2.2.3 BDNF Levels and Polymorphism Assessment

Blood samples were obtained between 10AM and 4PM, and serum (which does not contain platelets) was stored at -80C (between 5 and 60 months with no freeze-thaw cycles) until serum BDNF levels were measured using a high-sensitivity (<20pg/mL) and specific (no cross-reactivity with other growth factors, except 13% cross-reactivity with pro-BDNF) quantitative enzyme immunoassay (ELISA) (R&D Systems, Minneapolis, MN). There was no relationship between storage time and BDNF measures. All samples were measured in duplicate and the average intra-assay and inter-assay coefficients of variation were 6.2% and 11.3%, respectively. Of note, BDNF serum levels are not greatly associated with diurnal circadian rhythms[124] nor with platelet counts[125].

Genomic DNA isolated from lymphocytes (QuickGene-Mini-80 kit; Fujifilm Life Science; www.autogen.com) was assessed using the 5'-nuclease Taqman assay (ABI 7900 DNA detection system), employing Assays-on-Demand and Assays-by-Design (Applied Biosystems, Inc., Foster City, CA) with >95% accuracy. Although the BDNF polymorphism was in Harvey-Weinberg equilibrium, only three subjects were homozygous for Met/Met, which were therefore combined with the 38 Met/Val heterozygotes. Val/Val homozygotes were thus compared with any subject carrying the “lower secreted” Met allele.

2.2.4 Statistical Analysis

All statistics employed SPSS 18.0, and results are reported as mean ± standard deviation, and in graphs as mean ± standard error of the mean. Repeated-measure mixed-effect analyses, robust to randomly missing data (many subjects did not complete all assessments at all-time points), were used to compare symptom changes over time. For these mixed-effect models, we first examined repeated covariance structures, selecting analyses which provided the smallest Aikake Information Criteria (typically this was an unstructured covariance).

2.3 Results

2.3.1 Clinical and Demographic Characteristics of Subjects

The relationship between baseline characteristics of subjects and both BDNF variant allele carriers, and baseline BDNF levels are shown in Table 2. Subjects in this study were primarily middle aged (but ranged from 18 to 72 years), about 2/3 were male and mostly Caucasian, and almost 20% had a prior history of MDD in remission. All subjects starting IFN-α therapy had a Cumulative Illness Rating Scale-Geriatric (CIRS-G) score of at least 2 (because of HCV infection). Most subjects typically had only a few other medical problems diagnosed and treated such as hypertension and hyperlipidemia (e.g., less than 2% were being treated with statins) and 64% had CIRS-G scores of 4 or less. There was a trend for older subjects to have lower baseline BDNF levels, but other demographics were not correlated with baseline BDNF levels. Unless stated otherwise, we therefore did not include these variables as covariates in the analyses below.

Table 2: Relationship between baseline characteristics of subjects and both BDNF polymorphism and baseline BDNF levels

Variables	BDNF Genotype		Baseline BDNF levels	
	Val/Met and Met/Met (Mean±SD)	Val/Val (Mean±SD)	Correlation (r)	P-value
Age (years)	50.8±11.7	47.1±11.5	0.17	0.09
Gender (%Female)	35%	26.50%	0.08	ns
Race (% Caucasian)	87.80%	87.40%	0.06	ns
Weight (Kg)	82.5±18.1	86.6±6.5	0.08	ns
Sustained Viral Response	47%	45%	0.2	0.1
History of MDD	19.50%	18.80%	0.1	ns
BDNF (ng/mL)	17.0±10.5	19.3±10.0	1	NA
CIRS-G	4.5±2.4	3.7±1.6	0.1	ns
BDI	8.1±6.1	8.6±9.0	0.1	ns

BDNF= brain-derived neurotrophic factor; CIRS-G=Cumulative Illness Rating Scale-Geriatric; BDI-II=Beck Depression Inventory; MDD=Major Depressive Disorder; ns=not significant (p>0.15); (r)= Pearson correlation.

2.3.2 Relationship between Baseline BDNF Levels and Development of Depression

In our study, we observed that lower baseline BDNF was associated with increased BDI-II symptoms over time during IFN-α therapy ($F_{144,17.2}=6.8$; $p<0.0001$). This supports the hypothesis that baseline BDNF may be inversely related to subsequent depression vulnerability. We next included baseline BDI-II as a covariate because of its known association with subsequent depression risk. The association between BDNF and subsequent BDI-II over time was still significant ($F_{118,17.4}=2.0$; $p=0.05$).

2.3.3 Relationship Between Baseline Median BDNF Levels and Development of Depression

The relationship between baseline median BDNF levels and BDI-II scores are shown in Figure 1. To better characterize and illustrate our previous findings, we divided baseline BDNF levels by a median split (below or above 17 ng/mL). Compared with higher baseline BDNF levels, lower baseline levels (BDNF < 17 ng/mL) were associated with higher BDI-II scores ($F_{1,98.9}=4.7$; $p=0.03$). However, after controlling for baseline BDI-II, dichotomized baseline BDNF was associated with increasing BDI-II scores over time ($F_{4,46.2}=2.7$; $p=0.04$). These results are consistent with low BDNF levels being a moderator of subsequent vulnerability.

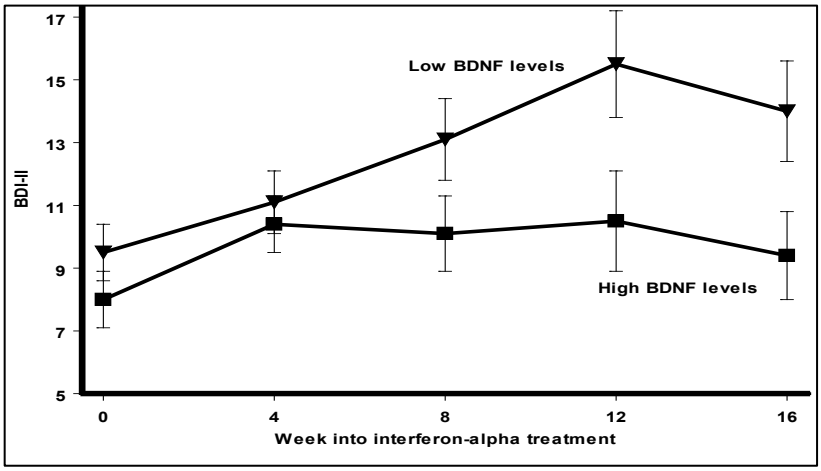


Figure 1: Relationship between baseline median BDNF levels and development of depression during IFN-α therapy.

This figure shows BDI-II scores in patients with low median baseline BDNF levels (black triangles) and high median baseline BDNF levels (black squares) overtime. BDI-II data are expressed as mean ± SEM. Data were compared using repeated measure mixed model. Statistical difference established at $p<0.05$.

2.3.4 Relationship Between Serum BDNF Levels and Development of Depression

The relationship between serum BDNF levels and the development of depression are shown in Figure 2. During IFN-α treatment, serum BDNF levels reduced over time in most subjects ($F_{4,37.7}=5.0$; $p=0.003$). Nonetheless, BDNF levels decreased similarly in the subjects who developed MDD and those who completed treatment ($F_{4,47.7}=0.6$; $p=0.6$). Therefore, although IFN-α treatment appears to decrease BDNF, these findings do not support the hypothesis that simply decreasing peripheral BDNF during IFN-α therapy is necessarily associated with the emergence of depression.

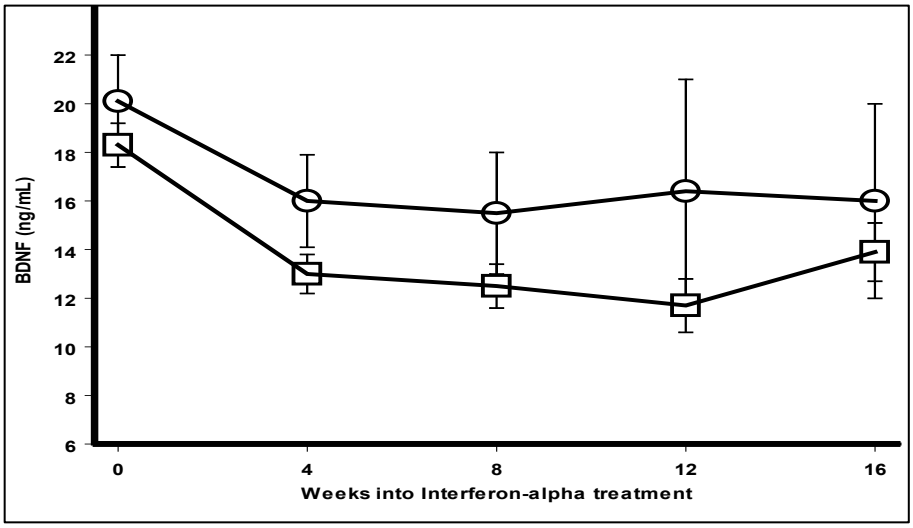


Figure 2: Relationship between serum BDNF levels and the development of depression during IFN-α therapy. This figure shows serum BDNF levels overtime in patients who develop MDD (open squares) and patients who did not develop MDD (open circles). Serum BDNF levels are expressed as mean ± SEM. Data were compared using repeated measure mixed model. Statistical difference established at $p<0.05$.

2.3.5 Comparison of Serum BDNF levels in BDNF Gene Variant Groups

The comparison of serum BDNF levels in BDNF gene variant groups is shown in Figure 3. Our study demonstrates that subjects with Val/Val genotype had higher serum BDNF levels ($F_{1,83.0}=5.0$; $p=0.03$) overtime when compared to subjects carrying the Met allele, but BDNF levels reduced similarly in both genetic groups ($F_{4,42.9}=0.3$; $p=0.9$).

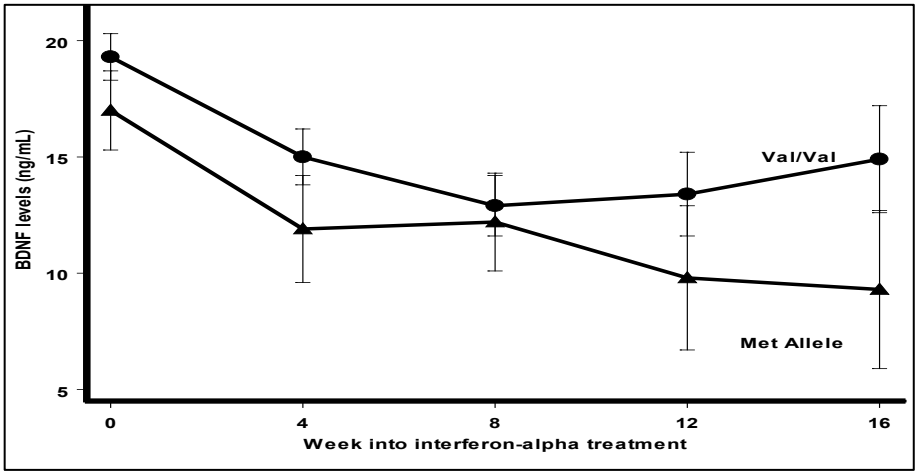
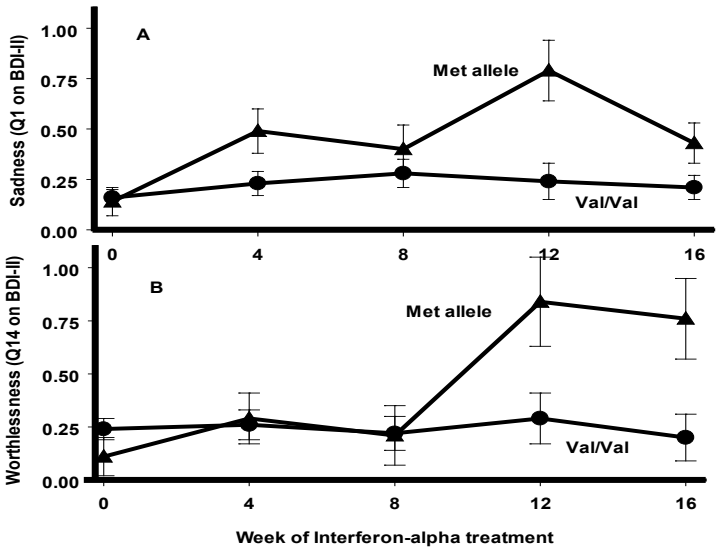


Figure 3: Comparison of serum BDNF levels in BDNF gene variant group

This figure shows serum BDNF levels in patients with Val/Val carriers (black circles) and Met allele carriers (black triangles) overtime. BDNF levels data are expressed as mean ± SEM. Data were compared using repeated measure mixed model. Statistical difference established at $p<0.05$.

2.3.6 Relationship between BDI-II items and BDNF Gene Variant Groups

Since we did not observe an association between BDNF gene variant groups and BDI-II scores, we investigated specific questions from the BDI-II assessment. We report that the Met allele was associated with increased psychological symptoms on the BDI-II, including sadness (Q1;$F_{4,21.2}=3.2$; $p=0.03$), and worthlessness (Q14; $F_{4,56.1}=2.7$; $p=0.04$) (Figure 4A and 4B). Also, the Met allele was associated with increased suicidal ideation (Q9 of the BDI-II; $F_{4,112.2}=2.5$; $p<0.05$) (Figure 4C). Notably, suicidal thoughts were uncommon – only 6.3% of people with Met allele and 3.2% of those with Val/Val answered "I have thoughts of killing myself, but I would not carry them out" on BDI-II question 9 during IFN-α treatment (and there were no suicide attempts by any participants during this study). Conversely, there was no BDNF genetic association with emergence of any neurovegetative symptoms such as insomnia (Q16; $F_{4,47.1}=0.7$; $p=0.6$), fatigue (Q20; $F_{4,44.2}=0.4$; $p=0.8$), nor appetite (Q18; $F_{4,39.9}=1.8$; $p=0.14$).



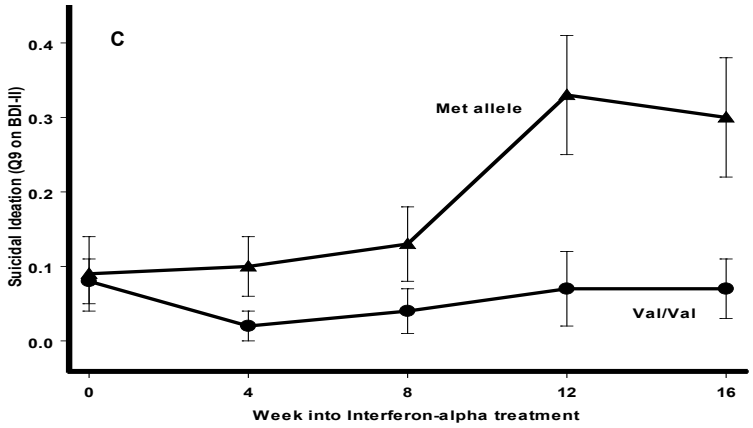


Figure 4: Relationship between BDNF polymorphism and psychological symptoms of depression

This figure shows the relationship between BDNF polymorphism and psychological symptoms of depression such as sadness (Panel A), worthlessness (Panel B), suicidal ideation (Panel C). Patients with Val/Val and Met allele carriers are represented by black circles and black triangles, respectively. BDI-II questionnaires are expressed as mean ± SEM. Data were compared using repeated measure mixed model. Statistical difference established at $p<0.05$.

2.4 Discussion

IFN-α therapy results in decreasing BDNF levels along with worsening depression scores, similar to a prior report of 17 patients in the Netherlands.[126] In support of a moderator hypothesis, lower BDNF levels prior to IFN-α therapy were predictive of greater depression symptoms during IFN-α treatment, even when controlling for baseline BDI-II scores. However, conclusions regarding a mediator hypothesis were more equivocal. BDNF decreased both in those who developed depression and those who did not, which is consistent with IFN-α having behavioral effects in rodents without affecting cortical BDNF levels.[127] Other studies support a moderating effect of BDNF on depression risk in other contexts. Lower BDNF (and/or the BDNF Met allele)

increases risk for depression symptoms in rhesus macaques exposed to early adversity[64], in humans exposed to stress[109, 128, 129], and in humans developing depression in the context of alcohol dependence[130] or Alzheimers disease[131].

There are several plausible pathways by which BDNF could moderate the effects of an inflammatory cytokine like IFN-α. First, inflammation could affect phosphorylation of BDNF's receptor (TrkB), interfering with BDNF signaling[68], and subsequent intracellular signal transduction can be impaired by inflammatory cytokines such as IL-1.[67] Both processes could impact depression more in those who start out with low BDNF. Second, BDNF and inflammatory cytokines both influence serotonin transporter transcription and function, likely through mitogen activated protein (MAP) kinase pathways. [132-135] IFN-α increases serotonin transporter transcription via the MAP kinase intracellular signaling pathway [136], and both BDNF and inflammatory cytokines share overlapping intracellular signal transduction pathways including MAP kinases [137] and nuclear factor Kappa-light-chain-enhancer of activated B cells (NF-kappaB). [138]Third, the BDNF Met allele has also been associated with an elevated cortical response to a dexamethasone/corticosterone releasing hormone challenge test[139], which is notable given that there is a greater cortical response to the initial injection of IFN-α in those at increased risk for subsequent depression.[60] Likely mediated by low BDNF levels, the Met allele was predictive of depression symptoms–an effect that was likely mediated by lower BDNF levels. Also, similar to prior reports [95-97], we specifically found that the Met allele was associated with increased suicide ideation, along with increased sadness and a sense of worthlessness. The Met allele was not associated with enhanced fatigue, insomnia, or appetite complaints. Thus, how one measures depression matters. In fact, different depression symptoms may be influenced by different genes[119],

a phenomenon long noted in mice where different chromosomal regions are implicated in anxiety-depending on what behavior test is employed.[118] The possibility that the Met allele is only associated with risk for a subset of symptoms may be one plausible reason that some studies do not replicate an association between depression risk and the BDNF Val/Met polymorphism.[111-116] There may also be treatment implications. The BDNF Met allele could be associated with better response to SSRIs[130, 131, 140], and suicidal ideation is the least common residual symptom following SSRI treatment. [141]

2.5 Conclusion

In this study, we investigated the relationship between both serum BDNF levels and Val66Met polymorphism, and the development of MDD in HCV patients on INF-α therapy. We report that lower baseline BDNF levels was associated with higher depression symptoms during IFN-α treatment. Also, Met allele was associated with lower BDNF levels, however it was not associated with increased BDI-II. An exploratory comparison of individual BDI-II items indicated that the Met allele was associated with suicidal ideation, sadness, and worthlessness, but not neurovegetative symptoms. In addition, we observed that IFN-α therapy further decreased BDNF serum levels, but this decrease occurred regardless of depression development and of genotype. These findings support the hypothesis that increased BDNF improves resiliency against developing inflammatory cytokine-associated depression, and specifically to a subset of symptoms.

3.0 Relationship Between Venlafaxine Dose and Drug Concentration

3.1 Introduction

Venlafaxine is a commonly used pharmacotherapy in the treatment of MDD. However, venlafaxine has been reported to exhibit high variability in drug concentration or PK parameters at steady-state. For example, clinical trials investigating venlafaxine PK reported high variability in steady-state plasma clearance of both venlafaxine and its metabolite ODV in MDD patients.[29] These studies also showed that hepatic and renal impairment significantly reduced the drug clearance and increased the drug exposure, thus dose adjustment is recommended in patients with these comorbidities.[29] In a similar fashion, it has been reported that patients on the same dosing regimen of certain psychotropic drugs, such as venlafaxine, demonstrate highly variable PK leading to under- or overdosage in 30–50 % of patients.[142] Based on the large inter-individual variability (IIV) in PK observed with venlafaxine treatment, some investigators have proposed titrating the dose using TDM to achieve a target therapeutic concentration range of 195 ng/ml to 400 ng/ml.[143,144] This proposed therapeutic concentration range has been noted as a level 2 recommendation in the AGNP-TDM Expert Group Consensus Guidelines: Therapeutic Drug Monitoring in Psychiatry.[143] However, TDM of venlafaxine is not currently recommended in the product label or the Practice Guideline for the Treatment of Patients With Major Depressive Disorder.[145]

In addition, a few clinical studies have investigated the relationship between venlafaxine dose and drug concentration in depressed patients. Interestingly, the correlation between

venlafaxine dose and drug concentration was reported as weak[142, 144] or moderate[146]. Some investigators have suggested that the dose of venlafaxine may not necessarily be the only predictor of plasma concentration because other factors, including age, sex, physiological factors, and drug–drug interactions, may influence the plasma drug concentration achieved at a given drug dose.[147] Collectively, these findings suggest that dose may not be highly predictive of drug concentration in MDD patients possibly due to the high variability in the PK of venlafaxine.

Thus, a better understanding of the relationship between venlafaxine dose and concentration along with the factors contributing to the variability in PK of venlafaxine may improve the treatment efficacy, reduce the treatment time required to achieve a clinically significant response, and potentially reduce the number and severity of adverse events associated with treatment in MDD patients. Given the clinical importance for identifying the sources of variability in venlafaxine PK, the goal of this chapter is to investigate the relationship between venlafaxine dose and drug concentration in MDD patients and to identify the impact of certain demographic and clinical factors on those relationships. Based on previously reported literature, we expect that there will be a large IIV in drug concentration even among patients with similar dosing regimens and that venlafaxine dose will not be strongly correlated with drug concentration across our patient population.

3.2 Materials and Methods

3.2.1 Study Design and Participants

The study protocol was approved by the Institutional Review Board at the University of Pittsburgh and informed consent was obtained from the patients or their care giver. The inclusion criteria included subjects diagnosed with MDD, single or recurrent, according to DSM-IV with baseline depression severity of at least moderate intensity as measured using MADRS (MADRS ≥15). Subjects were excluded from the study if they were diagnosed with bipolar disorder, schizophrenia, or other psychotic disorders, dementia or neurodegenerative diseases with known effects on mood, history of alcohol or substance abuse, high risk of suicide, unstable medical illness, and contraindication to venlafaxine treatment. Study participants were recruited from primary care; especially mental health sectors or self-referral in response to advertisement in the media/website (study website: latelifedepression.org and clinical trial.gov). A total of 57 participants signed consent but three subjects were excluded due to faster titration protocol (N=2) and inaccurate dosing record (N=1). Thus, 54 subjects were included in this analysis.

3.2.2 Dosing Regimen

Venlafaxine extended-release (Effexor XR®) dose was initiated at 37.5mg and titrated in 37.5mg increments at least every 3-4 days to a target dose of 150 mg/day. At the midpoint of study duration (week 6), subjects that did not achieve clinical response, defined as a decrease of ≥50% from baseline MADRS score, had their dose increased in 37.5-75mg increments every 3-4 days as tolerated to achieve a target dose of 300 mg/day.

3.2.3 Sampling and Analytical Methods

Blood samples were obtained at day 1, week 1 and week 12 post-treatment and were processed and stored at -80C. Plasma venlafaxine and ODV levels were measured using a validated high-performance liquid chromatography assay with tandem mass spectrometry (HPLC-MS/MS) method as previously described.[35] All the samples were analyzed in duplicate and the lower limits of quantification were 10ng/ml for both venlafaxine and ODV.

3.2.4 Patient Demographics and Baseline Clinical Characteristics

We assessed patient demographics including age, sex (male/female), race (Caucasian/ African American) and clinical characteristics including comorbid medical burden as measured by cumulative illness rating scale for geriatric (CIRS-G), hepatic disease (yes/no), renal disease (yes/no), weight (wt), and body mass index (BMI). BMI was calculated as follows:$703 \times \frac{wt}{ht^2}$.

3.2.5 Statistical Analysis

Statistical analyses were performed using IBM SPSS statistical software version 24.0 (SPSS Inc., Chicago, IL, USA). In this study, we assessed the relationship between venlafaxine dose and total drug concentration. Venlafaxine dose was assessed as the last dose, also referred to as the end dose, at week 1 and week 12 or dose trajectory pattern over time. The group-based trajectory analysis was performed using PROC TRAJ software package in SAS version 9.4.[148] As previously reported, total drug plasma concentration was obtained by combining the plasma levels of both venlafaxine and ODV at day 1, week 1, and week 12. The percentage of patients within

the proposed target therapeutic range (195 to 400 ng/ml) was determined at week 1 and week 12. The non-detectable plasma concentration levels were recorded as lower limit of quantification (LLQ)/2. Missing samples were not imputed. Dose-corrected concentration was calculated by normalizing the total concentration by end dose at week 1 and week 12. Covariates included in the regression analysis included patient demographics and baseline clinical characteristics. Age, weight, BMI, and CIRS-G were evaluated as continuous variables. Gender, race, hepatic disease, and renal disease were evaluated as binary variables.

3.2.5.1 Data Distribution

We assessed the normality of our potential predictor variables using Kolmogorov-Smirnov tests with a p-value < 0.05 indicating a non-normal distribution. Variables which demonstrated non-normal distribution were evaluated using non-parametric analyses.

3.2.5.2 Test of Association

The association between two categorical variables was assessed using the chi-square test or Fisher's exact test if any group showed a frequency of five or less. A comparison between two groups was performed using the student's t-test and Mann-Whitney U-test for normally and non-normally distributed data, respectively. A comparison between more than two groups was performed using ANOVA with Bonferroni post hoc test and Kruskal Wallis test for normally and non-normally distributed data, respectively. The association between two continuous variables was evaluated using Pearson and Spearman correlation for normally and non-normally distributed

variables, respectively. Two-tailed p-values below 0.05 were regarded as statistically significant in all analyses.

3.2.5.3 Regression Analysis

We evaluated the effect of venlafaxine dose on drug concentration after adjusting for covariates using regression analysis. Logistic regression and linear regression were used when evaluating categorical and continuous variables, respectively. Linear regression was used for continuous outcomes, total drug concentration at week 1 and week 12. For logistic regression analysis, we reported the odds ratio (OR) and 95% confidence interval (CI). For linear regression, we reported standardized regression coefficient and 95% CI.

(a) Linear Regression

We used linear regression analysis to evaluate the relationship between venlafaxine dose and drug concentration at week 1 and week 12 after adjusting for covariates. In the linear regression analysis, we included covariates which demonstrated a trend for a significant association ($p<0.10$) with drug concentration. We also included certain covariates which have been previously reported to be significantly associated with drug concentration even if these variables were not statistically significant in our analyses.[149]

All potential predictors variables were then entered into a backward stepwise linear regression model to select the smallest subset of variables that predict changes in drug concentration at week 1 and week 12. Variables entered into the model included age, gender, hepatic disease, renal disease, weight, BMI. Model entry and exit p-values were set at 0.10 and

0.20, respectively. These entries and exit p-values were chosen to prevent potentially important predictors from being excluded. While liberal p-values were chosen for selecting the final set of independent variables, we considered only those variables with $p<0.05$ as statistically significant. After defining our model, we checked whether the assumption for the models was met by plotting a graph of the residuals vs. the predicted values to demonstrate the normality and constant variance of the residuals.

(b) Logistic Regression

A logistic regression was used to explore the relationship between predictors variables and achieving the target therapeutic range (3 groups: within/ above/below the target therapeutic range). The same method described for covariate selection and model building for the linear regression were used to develop logistic regression. Briefly, potential predictors were tested using univariate analysis. Potential predictors variables with a p-value ≤ 0.2 in the univariate analyses were included in a backward multivariate logistic regression procedure to evaluate which variables were independently associated with the likelihood of achieving target therapeutic range.[150] The regression models were evaluated for goodness of fit using the Hosmer and Lemeshow test with receiver operating characteristic curve (ROC).[151]

3.3 Results

3.3.1 Description of Venlafaxine Dosing Regimen

3.3.1.1 Description of Venlafaxine Dosing Overtime

The venlafaxine dosing regimen during the 12-week study is summarized in Figure 5. Based on the study protocol, the dose was titrated to higher strengths over time, especially in patients that did not respond at week 6. About 80% of patients taking venlafaxine reach the target dose (150 mg/day) after two weeks of starting venlafaxine treatment. After week 6, ~40% of patients were maintained on a dose of ≤150 mg/day and the remaining patients were titrated to higher doses. At the end of the study, the mean dose was 220.1± 69.6 mg/day (range: 112.5-300 mg/day) and ~40% of patients were receiving the maximum dose of 300 mg/day.

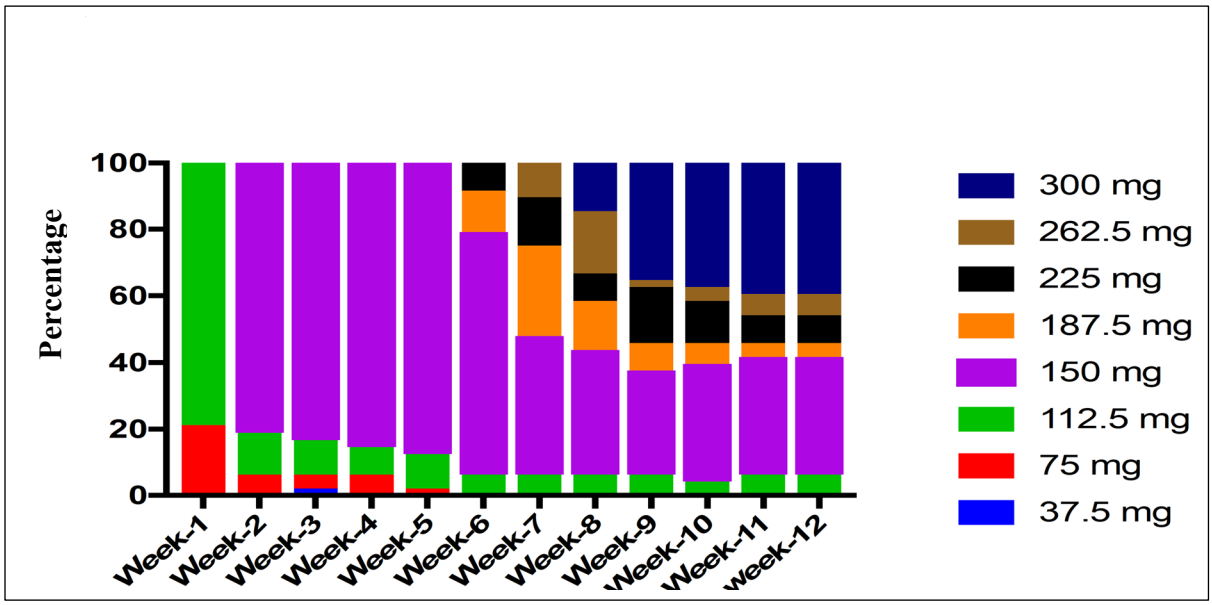


Figure 5: Venlafaxine dosing regimen during the 12-week study

3.3.1.2 Description of Venlafaxine Dose Trajectory Patterns

The venlafaxine dose profiles and trajectory patterns are shown in Figure 6. The trajectory model evaluating venlafaxine dose show two groups of patients with significantly different dose profiles from week 1-12 after starting venlafaxine treatment (P<0.001). The dose values in trajectory groups are presented as mean with 95% confidence interval. Patients in the "low" group (n=23, 46.9%) have a relatively low mean dose over the entire study duration. Patients in the "high" group (n=25, 53.1%) have relatively low mean dose from week 1 to 5 followed by a significant dose titration from weeks 6 to 8 reaching a plateau from weeks 9 to 12.

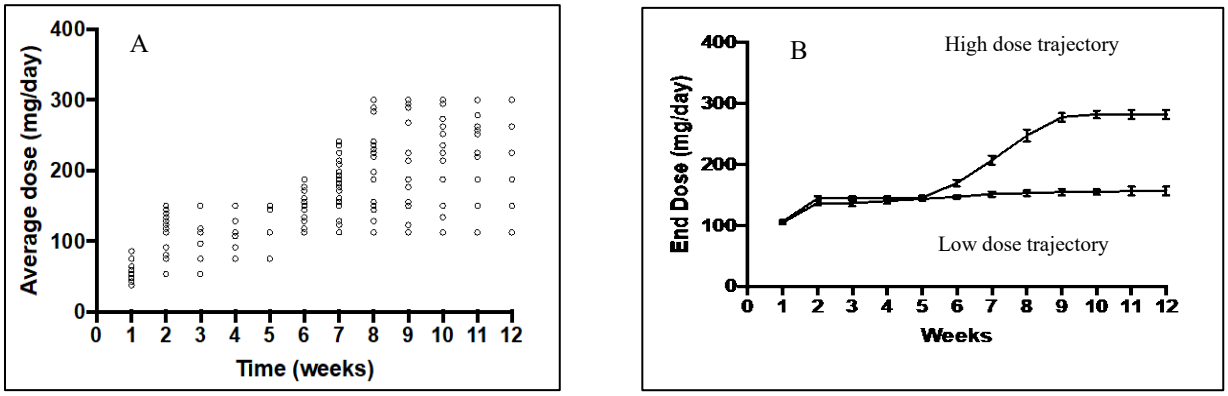


Figure 6: Venlafaxine dosing profile

Panel (A) shows raw population values of average venlafaxine dose (mg/day) from 54 patients up to 12 weeks after starting venlafaxine treatment. Panel (B) shows the venlafaxine dose versus time from starting treatment (weeks) in high {filled triangles, n=25 (53.1%)} and low {filled circles, n=23 (46.9%)} groups as identified by trajectory analysis. Dose data are presented as mean with 95% confidence interval.

3.3.2 Description of Drug Concentration

3.3.2.1 Description of Drug Concentration at Week 1 and Week 12

Results showing the distribution of drug concentration values at week 1 and week 12 are shown in Figure 7, Figure 8 and Table 3. The drug concentration values were normally distributed at week 1 and week 12. The mean ± SD (minimum to maximum) concentration levels at week 1 and week12 were 320.8±121.2 ng/ml (32-790 ng/ml) and 578.02±245.7 ng/ml (177-1060 ng/ml) respectively. In addition, we observed high variability (CV>30%) in drug concentration at week 1 and week 12.

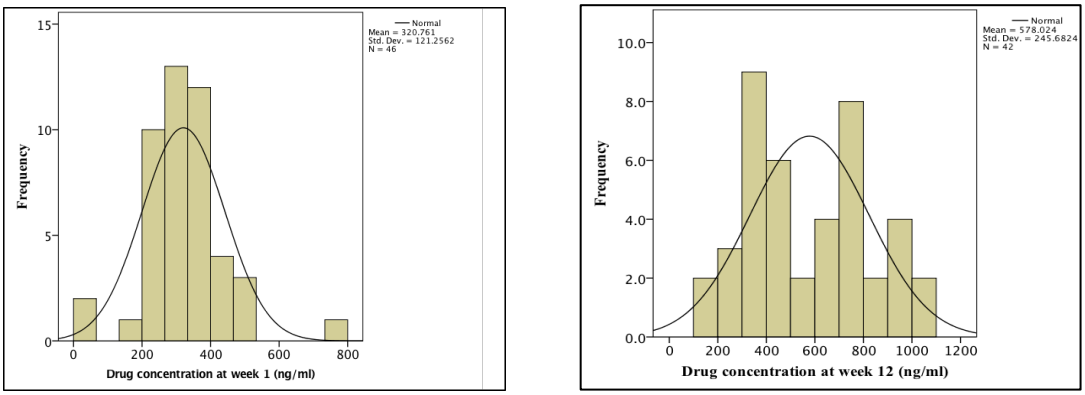


Figure 7: Histogram of drug concentration at week 1 and week 12

x-axis: drug concentration levels (ng/ml); y-axis: frequency of blood samples

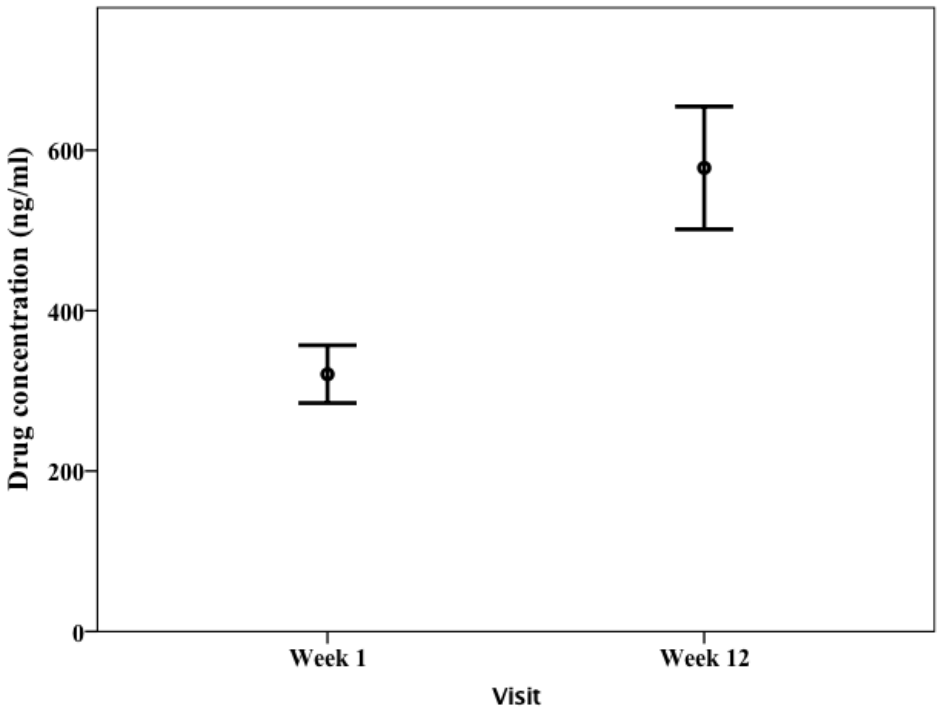


Figure 8: Drug conentration overtime

Drug concentration (ng/ml) from 54 patients at week 1 and week 12 after starting venlafaxine treatment. Drug concentration data are presented as mean ± standard error of mean (SEM). Statistical difference was established at *p<0.05.

Table 3: Descriptive of drug concentration at week 1 and week 12

Drug concentration (ng/ml)	n	Mean	SD	Median	Minimum	Maximum	CV%
Concentration at Week 1	46	320.761	121.256	308.5	32	790	37.8
Concentration at Week 12	42	578.024	245.682	565	177	1060	42.5

CV is the coefficient of variation and SD is the standard deviation.

3.3.2.2 Descriptive of Drug Concentration by Dose Trajectory

Results showing the drug concentration descriptive values in low and high dose trajectory group are shown in Table 4. The mean ± SD (minimum to maximum) concentration levels at week 1 and week12 in low dose trajectory group were 354.5±76.2 ng/ml (210-510ng/ml) and 437.8±217.6 ng/ml (177-1010 ng/ml), respectively. The mean ± SD (minimum to maximum) concentration levels at week 1 and week12 in high dose trajectory group were 278.3±108.9ng/ml (32-472ng/ml) and 705.5±198.2 ng/ml (374-1060 ng/ml), respectively. In addition, we observed high variability (CV>30%) in drug concentration at week 1 and week 12 among patients in the high and low dose trajectory group, respectively.

Table 4: Drug concentration at week 1 and week 12 by dose trajectory

Drug concentration (ng/ml)	N	Mean	SD	Median	Minimum	Maximum	CV%
Low dose trajectory group							
Concentration at week 1	20	354.5	76.2	343	210	510	21.5
Concentrationat week 12	20	437.8	217.6	375	177	1010	49.7
High dose trajectory group							
Concentration at week 1	22	278.3	108.9	290.5	32	472	39.1
Concentrationat week 12	22	705.5	198.2	735	374	1060	28.1

CV is the coefficient of variation and SD is the standard deviation.

3.3.2.3 Dose-Corrected Drug Concentration

Results showing dose-corrected drug concentration distribution and descriptive are shown in Figures 9 and Table 5. The dose-corrected concentration values were normally distributed at week 1 and week 12. The mean and maximum dose-corrected concentration levels at week 1 and week12 were 2.92±1.07ng/ml/day (0.28-7.02ng/ml/day), 2.55±0.819 ng/ml/day (1.18 4.98ng/ml/day), respectively. In addition, we observed high variability (CV>30%) in the dose-corrected drug concentration at week 1 and week 12.

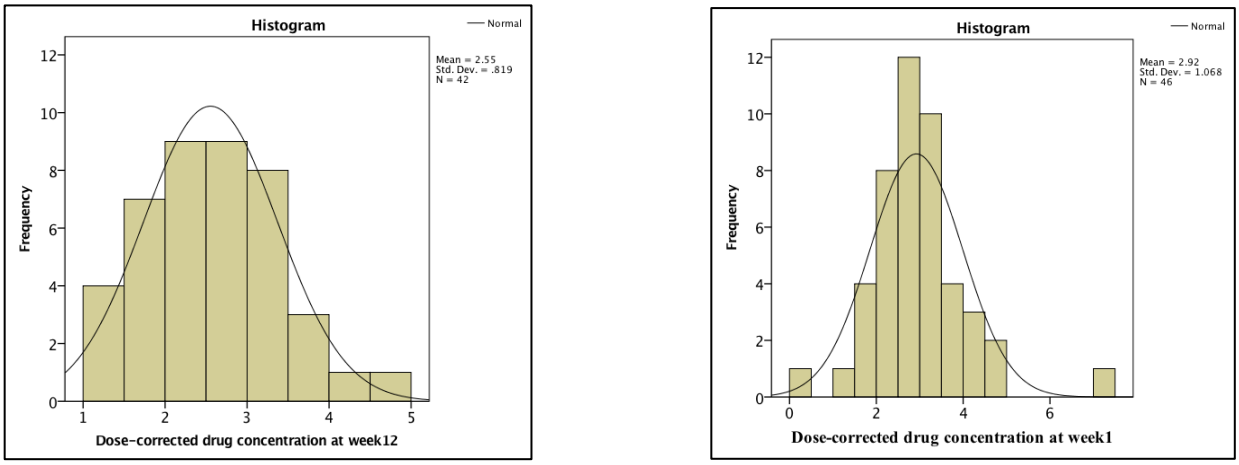


Figure 9: Histogram of dose-corrected concentration at week 1 and week 12

x-axis: dose-corrected drug concentration levels (ng/ml); y-axis: frequency of blood samples

Table 5: Descriptive of dose-corrected concentration at week 1 and week 12

Dose-corrected concentration (ng/ml/ml)	n	Mean	SD	Median	Minimum	Maximum	CV%
Concentration at Week 1	46	2.92	1.068	2.74	0.28	7.02	36.6
Concentration at Week 12	42	2.55	0.819	2.53	1.18	4.98	32.1

CV is the coefficient of variation and SD is the standard deviation.

3.3.2.4 Venlafaxine Target Therapeutic Concentration Range

The percentage of patients within the target therapeutic range (195 to 400 ng/ml) at week 1 and week 12 is shown in Figure 10. At week 1, approximately 78%, 17% and 4% of patients were within, above, and below the therapeutic range, respectively. At week 12, approximately 66% and 33% of patients were above and within the therapeutic range, respectively.

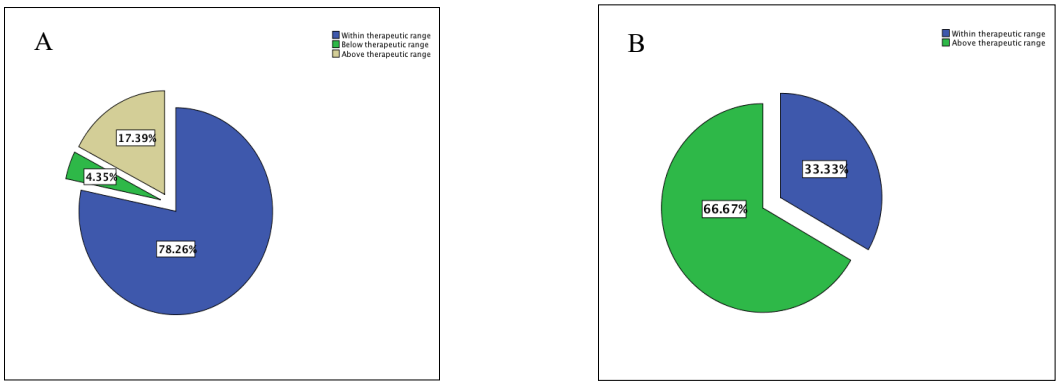


Figure 10: Pie charts showing the percentage of patients within, below and above the therapeutic range

The proposed therapeutic range for venlafaxine is 195 to 400ng/ml. Panel A shows the percentage of patients within, below and above the proposed therapeutic range at week 1. Panel B shows the percentage of patients within and above the proposed therapeutic range at week 12. The blue, green, and brown colors represent the percentage of patients within, below, and above the proposed therapeutic range, respectively.

3.3.3 Relationship Between Covariates and Dose

3.3.3.1 Relationship Between Covariates and End Dose

The relationship between covariates and end dose at week 1 and week 12 is shown in Table 6 and Table 7. There were no statistically significant associations between covariates and end dose at week 1 and week 12.

Table 6: Relationship between covariates and end dose at week 1

Categorical Covariates			
Covariate	**Group**	**End dose at week 1**	
		Mean ± SD (n)	**P-value**
Age	≤ 65	109.5± 18.49 (25)	0.735
	> 65	109.5± 15.0(25)	
Gender	Male	110.53± 8.60(19)	0.868
	Female	108.87± 20.21(31)	
Race	Caucasian	107.93± 14.99(41)	0.383
	African American	116.67± 22.53(9)	
Hepatic Disease	No	108.65± 18.84(39)	0.825
	Yes	112.5± 0.00(4)	
Renal Disease	No	109.29± 19.02(35)	0.818
	Yes	107.81± 13.26(8)	
Upper-GI Disease	No	107.39± 21.00(22)	0.691
	Yes	110.71± 14.41(21)	
Lower-GI Disease	No	109.72± 20.61(27)	0.531
	Yes	107.81± 12.81(16)	
Continuous Covariates			
Covariate	**n**	**End dose at week 1**	
		Spearman Correlation (rs)	**P-value**
Age (yr)	50	-0.138	0.338
Weight (lb)	50	0.128	0.376
BMI	50	0.078	0.588

Categorical and continuous covariates were compared to end dose at week 1 using Mann-Whitney U-test and Spearman correlation, respectively. GI= Gastrointestinal, yr=year, lb=pounds. Statistical difference was established at *p<0.05

Table 7: Relationship between covariates and end dose at week 12

Categorical Covariates			
Covariate	**Group**	**End dose**	
		Mean ± SD (n)	**P-value**
Age	≤ 65	223.37 ± 71.93(23)	0.712
	> 65	220.31 ± 69.33(24)	
Gender	Male	246.09 ± 69.78(16)	0.106
	Female	209.27 ± 67.62(31)	
Race	Caucasian	220.07± 70.38(38)	0.642
	African American	229.17 ± 71.26(9)	
Hepatic Disease	No	216.43± 71.66(35)	0.279
	Yes	255.00 ± 67.08(5)	
Renal Disease	No	223.89 ± 71.79(34)	0.754
	Yes	206.25 ± 74.06(6)	
Upper-GI Disease	No	221.25 ± 75.883(20)	0.862
	Yes	221.25 ± 68.72(20)	
Lower-GI Disease	No	207.00 ± 70.26(25)	0.192
	Yes	245.00 ± 69.243(15)	
Continuous Covariates			
Covariate	**n**	**End dose**	
		Spearman Correlation (rs)	**P-value**
Age (yr)	47	-0.117	0.434
Weight (Ib)	47	-0.07	0.639
BMI	47	-0.237	0.108

Categorical and continuous covariates were compared to end dose at week 12 using Mann-Whitney U-test and Spearman correlation, respectively. GI= Gastrointestinal, yr=year, lb=pounds. Statistical difference was established at *p<0.05.

3.3.3.2 Relationship Between Covariates and Dose Trajectory

The relationship between covariates and dose trajectory patterns are shown in Table 8. Males were more likely to fall in the high dose trajectory group when compared to females {OR (90% CI) = 0.268 (0.070-1.020), p=0.047}.

Table 8: Relationship between covariates and dose trajectory

Categorical Covariates						
Covariate	**Group**	**n**	**Week 12**			
			Low dose trajecotry	**High dose trajectory**	**OR (95% CI)**	**P-value**
			n (%)	**n (%)**		
Age	≤ 65	48	11 (45.8%)	13 (12.5%)	0.846 (0.272-2.629)	0.773
	> 65		12 (50%)	12 (50%)		
Gender	Male	48	4 (26.7%)	11 (73.3%)	0.268 (0.070-1.020)	0.047
	Female		19 (57.6%)	14 (42.4%)		
Race	Caucasian	48	18 (46.2%)	21 (53.8%)	0.686 (0.160-2.946)	0.719
	African American		5 (55.6%)	4 (44.4%)		
Hepatic Disease	No	41	20 (55.6%)	16 (44.4%)	5 (0.507-49.266)	0.184
	Yes		1 (20%)	4 (80%)		
Renal Disease	No	41	18 (51.4%)	17 (48.6%)	1.059 (0.187-5.985)	0.645
	Yes		3 (50%)	3 (50%)		
Upper-GI Disease	No	41	10 (50%)	10 (50%)	0.909 (0.267- 3.096)	0.879
	Yes		11 (52.4%)	10 (47.6%)		
Lower-GI Disease	No	41	15 (57.7%)	11 (42.3%)	2.045 (0.561-7.455)	0.341
	Yes		6 (40%)	9 (60%)		
Continuous Covariates						
Covariate	**Group**	**n**	**Week 12**			
			Low dose trajecotry	**High dose trajectory**	**OR (95% CI)**	**P-value**
			Mean ± SD (n)	**Mean ± SD (n)**		
Age (yr)	NA	48	66.74 ± 7.424 (23)	65.64 ± 6.013 (25)	NA	0.574
Weight (lb)	NA	48	183.843 ± 49.1716 (23)	184.780 ± 27.6837 (25)	NA	0.936
BMI	NA	48	31.630 ± 7.1439 (23)	29.941 ± 4.6366 (25)	NA	0.342

Categorical and continuous covariates were compared to low and high dose trajectory using Chi-square test and Student's t-test, respectively. GI= Gastrointestinal, yr=year, lb=pounds. Statistical difference was established at p<0.05.

3.3.4 Relationship Between Covariates and Drug Concentration

3.3.4.1 Covariates and Drug Concentration

The relationship between covariates and drug concentration at week 1 and week 12 are shown in Table 9 and Table 10. At week 1, patients >65 years of age had higher drug concentration (368.7±115.8 ng/ml) when compared to patients ≤ 65 years of age (268.5±106.5 ng/ml) (p=0.004). Likewise, age shows a weak positive correlation with drug concentration (r=0.415, p=0.004) at week 1. At week 12, BMI shows a weak negative correlation with drug concentration (r= -351, p=0.023).

Table 9: Relationship between covariates and drug concentration at week 1

Categorical Covariates			
Covariate	**Group**	**Concentration at week 1**	
		Mean ± SD (n)	**P-value**
Age	≤ 65	268.46 ± 106.47(22)	0.004*
	> 65	368.71 ± 115.76(24)	
Gender	Male	304.17 ± 156.89 (18)	0.463
	Female	331.43 ± 93.29 (28)	
Race	Caucasian	321.25 ± 117.32 (40)	0.945
	African American	317.5 ± 157.95 (6)	
Hepatic Disease	No	334.49 ± 120.83 (37)	0.078
	Yes	217.25 ± 146.74 (4)	
Renal Disease	No	321.36 ± 136.22 (33)	0.865
	Yes	330.00 ± 80.64 (8)	
Upper-GI Disease	No	322.65 ± 148.35 (20)	0.985
	Yes	323.43 ± 105.27 (21)	
Lower-GI Disease	No	327.8 ± 148.96 (25)	0.768
	Yes	315.62 ± 83.99 (16)	
Continuous Covariates			
Covariate	**N**	**Concentration at week 1**	
		Correlation (r)	**P-value**
Age (yr)	46	0.415	0.004*
Weight (Ib)	46	-0.078	0.606
BMI	46	-0.038	0.803

Categorical and continuous covariates were compared to drug concentration week 1 using Pearson correlation and Student's t-test, respectively. GI= Gastrointestinal, yr=year, lb=pounds. Statistical difference was established at *p<0.05.

Table 10: Relationship between covariates and drug concentration at week 12

Categorical Covariates			
Covariate	**Group**	**Concentration at week 12 Mean ± SD (n)**	**P-value**
Age	≤ 65	538.75 ± 251.95 (20)	0.329
	> 65	613.73 ± 240.01 (22)	
Gender	Male	657.92 ± 162.85 (13)	0.095
	Female	542.21 ± 269.68 (29)	
Race	Caucasian	585.23 ± 236.49 (35)	0.676
	African American	542 ± 306.12(7)	
Hepatic Disease	No	565.39 ± 248.89 (31)	0.519
	Yes	643.4 ± 244.61 (5)	
Renal Disease	No	585.57 ± 240.38 (30)	0.618
	Yes	529.5 ± 294.14 (6)	
Upper-GI Disease	No	585.12 ± 247.37 (16)	0.849
	Yes	569.1 ± 251.71 (20)	
Lower-GI Disease	No	521.57 ± 267.81 (21)	0.117
	Yes	652.73 ± 196.23 (15)	
Continuous Covariates			
Covariate	**n**	**Concentration at week 12 Pearson (r)**	**P-value**
Age (yr)	42	0.062	0.698
Weight (lb)	42	-0.138	0.383
BMI	47	-0.351	0.023*

Categorical and continuous covariates were compared to drug concentration week 12 using Pearson correlation and Student's t-test, respectively. GI= Gastrointestinal, yr=year, lb=pounds. Statistical difference was established at *p<0.05.

3.3.4.2 Covariates and Dose-Corrected Concentration

The relationship between covariates and dose-corrected concentration at week 1 and week 12 are shown in Table 11 and Table 12. At week 1, patients >65 years of age had higher dose-corrected concentration (3.37±1.107 ng/ml/day) when compared to patients ≤ 65 years of age (2.431±0.786 ng/ml/day) (p=0.002). Likewise, age shows a moderate positive correlation with dose-corrected drug concentration (r=0.503, p<0.001) at week 1. Also, we observed a trend of lower dose-corrected drug concentration in patients with hepatic disease (1.931±1.304ng/ml/day) when compared to patients without hepatic disease (3.062±1.051 ng/ml/day) (p=0.052).

At week 12, we observed a trend of higher dose-corrected concentration (2.76±0.89 ng/ml/day) in patients > 65 years of age when compared to patients ≤ 65 years of age (2.31±0.68ng/ml/day) (p=0.072). Likewise, age shows a weak positive correlation with dose-corrected drug concentration (r=0.318, p=0.04) at week 12.

Table 11: Relationship between covariates and dose-corrected concentration at week 1

Categorical Covariates			
Covariate	**Group**	**Dose-corrected concentration at week 1**	
		Mean ± SD (n)	**P-value**
Age	≤ 65	2.431 ± 0.786(22)	0.002*
	> 65	3.373 ± 1.107 (24)	
Gender	Male	2.76 ± 1.39 (18)	0.418
	Female	3.026 ± 0.813 (28)	
Race	Caucasian	2.984 ± 1.049 (40)	0.320
	African American	2.513 ± 1.202 (6)	
Hepatic Disease	No	3.062 ± 1.051 (37)	0.052
	Yes	1.931 ± 1.304 (4)	
Renal Disease	No	2.924 ± 1.207 (33)	0.758
	Yes	3.062 ± 0.624 (8)	
Upper-GI Disease	No	2.917 ± 1.176 (20)	0.851
	Yes	2.984 ± 1.076 (21)	
Lower-GI Disease	No	2.923 ± 1.219 (25)	0.844
	Yes	2.994 ±0 .956 (16)	
Continuous Covariates			
Covariate	**n**	**Dose-corrected concentration at week 1**	
		Pearson (r)	**P-value**
Age (yr)	46	0.503	<0.001*
Weight (Ib)	46	-0.192	0.201
BMI	46	-0.12	0.429

Categorical and continuous covariates were compared to dose-corrected concentration week 1 using Pearson correlation and Student's t-test, respectively. GI= Gastrointestinal, yr=year, lb=pounds. Statistical difference was established at *p<0.05.

Table 12: Relationship between covariates and dose-corrected concentration at week 12

Categorical Covariates			
Covariate	**Group**	**Dose-corrected concentration at week 12**	
		Mean ± SD (n)	**P-value**
Age	≤ 65	2.31 ± 0.68 (20)	0.072
	> 65	2.76 ± 0.89 (22)	
Gender	Male	2.73 ± 0.96 (13)	0.352
	Female	2.47 ± 0.75 (29)	
Race	Caucasian	2.62±0.84 (35)	0.239
	African American	2.22±0.67(7)	
Hepatic Disease	No	2.59 ±0.88(31)	0.780
	Yes	2.48±0.49(5)	
Renal Disease	No	2.59±0.81(30)	0.816
	Yes	2.50±1.02(6)	
Upper-GI Disease	No	2.60±0.99(16)	0.863
	Yes	2.55±0.71(20)	
Lower-GI Disease	No	2.43±0.78(21)	0.240
	Yes	2.77±0.89(15)	
Continuous Covariates			
Covariate	**n**	**Dose-corrected concentration at week 12**	
		Pearson (r)	**P-value**
Age (yr)	42	0.318	0.04*
Weight (lb)	42	-0.086	0.59
BMI	42	-0.208	0.186

Categorical and continuous covariates were compared to dose-corrected concentration week 12 using Pearson correlation and Student's t-test, respectively. GI= Gastrointestinal, yr=year, lb=pounds. Statistical difference was established at *p<0.05.

3.3.4.3 Covariates and Proposed Target Therapeutic Drug Concentration

The relationship between covariates and proposed target therapeutic concentration range at week 1 and week 12 are shown in Table 13 and Table 14. There was no association between

covariates and proposed target therapeutic drug concentration at week 1. At week 12, male patients were 1.9-fold more likely to achieve drug concentrations above the proposed therapeutic range (p=0.002), respectively. Also, BMI is lower (28.5 ± 4.6) in patients above the proposed target therapeutic concentration range when compared to patients that achieved the proposed target therapeutic concentration range (33.4 ± 7.2, p=0.010).

Table 13: Relationship between covariates and proposed target therapeutic concentration range at week 1

Categorical Covariates						
Covariate	**Group**	**n**	**Week 1**			**P-value**
			Below Target Conc	**Within Target Conc**	**Above Target Conc**	
			n (%)	**n (%)**	**n (%)**	
Age	≤ 65	22	2 (9.1%)	18 (81.8%)	2 (9.1%)	0.092
	> 65	24	0 (0%)	18 (75%)	6 (25%)	
Gender	Male	18	1 (5.6%)	15 (83.3%)	2 (11.1%)	0.636
	Female	28	1 (3.6%)	21 (75%)	6 (21.4%)	
Race	Caucasian	40	1 (2.5%)	33 (82.5%)	6 (15.0%)	0.202
	African American	6	1 (16.7%)	3 (50.0%)	2 (33.3%)	
Hepatic Disease	No	37	1 (2.7%)	28 (75.7%)	8 (21.6%)	0.155
	Yes	4	1 (25.0%)	3 (75.0%)	0 (0.0%)	
Renal Disease	No	33	2 (6.1%)	25 (75.8%)	6 (18.2%)	0.603
	Yes	8	0 (0.0%)	6 (75.0%)	2 (25.0%)	
Upper-GI Disease	No	20	1 (5%)	15 (75%)	4 (20%)	0.996
	Yes	21	1 (4.8%)	16 (76.2%)	4 (19%)	
Lower-GI Disease	No	25	2 (8%)	17 (68%)	6 (24%)	0.206
	Yes	16	0 (0%)	14 (87.5%)	2 (12.5%)	
Continuous Covariates						
Covariate	**Group**	**n**	**Week 1**			**P-value**
			Below Target Conc	**Within Target Conc**	**Above Target Conc**	
			Mean ± SD (n)	**Mean ± SD (n)**	**Mean ± SD (n)**	
Age (yr)	NA	46	61.5 ± 2.12(2)	65.81 ±7.28(36)	70.75 ±6.84(8)	0.137
Weight (lb)	NA	46	166.00 ± 4.24(2)	180.85 ± 41.87(36)	171.70 ±16.36(8)	0.743
BMI	NA	46	27.07 ± 6.14(2)	29.81±6.17(36)	29.37 ± 5.39(8)	0.818

Categorical and continuous covariates were compared to proposed target therapeutic range at week 1 using

Chi-square and ANOVA test, respectively. GI= Gastrointestinal, yr=year, lb=pounds. Statistical difference was established at *p<0.05.

Table 14: Relationship between covariates and proposed target therapeutic concentration range at week 12

Categorical Covariates						
Covariate	**Group**	**n**	**Week 12**			
			Within Target Conc	**Above Target Conc**	**OR (95% CI)**	**P-value**
			n (%)	**n (%)**		
Age	≤ 65	20	8 (40%)	12 (60%)	1.778 (0.486-6.5)	0.382
	> 65	22	6 (27.3%)	16 (72.7%)		
Gender	Male	13	0 (0%)	13 (100%)	1.933 (1.360-2.748)	0.002*
	Female	29	14 (48.3%)	15 (51.7%)		
Race	Caucasian	35	10 (28.6%)	25 (71.4%)	0.300 (0.057-1.589)	0.197
	African American	7	4 (57.1%)	3 (42.9%)		
Hepatic Disease	No	31	11 (35.5%)	20 (64.5%)	2.2 (0.218-22.197)	0.646
	Yes	5	1 (20%)	4 (80%)		
Renal Disease	No	30	10 (33.3%)	20 (66.7%)	1 (0.156-6.420)	1.000
	Yes	6	2 (33.3%)	4 (66.7%)		
Upper-GI Disease	No	16	3 (18.8%)	13 (81.3%)	0.282 (0.061-1.307)	0.097
	Yes	20	9 (45%)	11 (55%)		
Lower-GI Disease	No	21	10 (47.6%)	11 (52.4%)	5.909 (1.061-32.915)	0.031*
	Yes	12	2 (13.3%)	10 (86.7%)		
Continuous Covariates						
Covariate	**Group**	**n**	**Week 12**			
			Within Target Conc	**Above Target Conc**	**OR (95% CI)**	**P-value**
			Mean ± SD (n)	**Mean ± SD (n)**		
Age (yr)	NA	42	65.00 ± 4.67 (14)	67.14 ± 7.27 (28)	NA	0.323
Weight (lb)	NA	42	184.10 ± 44.11 (14)	177.69 ± 31.12 (28)	NA	0.588
BMI	NA	42	33.41 ± 7.18 (14)	28.51 ± 4.59 (28)	NA	0.01*

Categorical and continuous covariates were compared to proposed target therapeutic range at week 12 using Chi-square and Student's t-test, respectively. GI= Gastrointestinal, yr=year, lb=pounds. Statistical difference was established at *p<0.05.

3.3.5 Relationship Between Dose and Drug Concentration

3.3.5.1 Dose and Drug Concentration

The relationship between venlafaxine end dose and dose trajectory with drug concentration at week 1 and 12 is shown in Figure 11 and Table 15. At week 1, there was no correlation between end dose and drug concentration. However, after controlling for covariates, end dose shows a weak positive correlation with drug concentration at week 1 (rs= 0.375, p=0.007). At week 12, the end dose shows a strong positive correlation with drug concentration at week 12 (rs= 0.758, p<0.001) before and after controlling for covariates. In addition, patients in the high dose trajectory group had higher drug concentration at week 12 (705.5 ± 198.2 ng/ml) when compared to patients in the low dose trajectory group (437.8 ± 217.6 ng/ml, p<0.001). This relationship did not change after controlling for covariates.

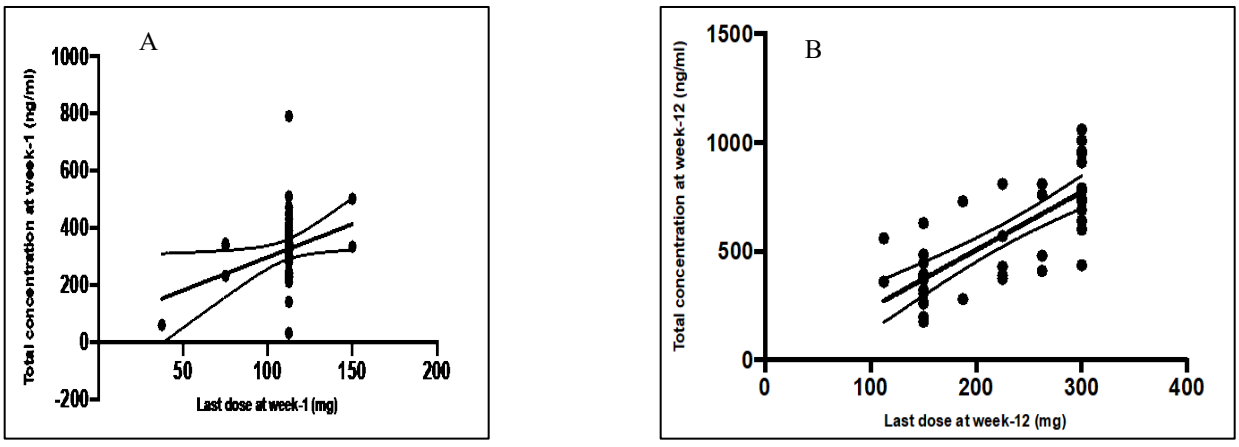


Figure 11: Correlation between venlafaxine dose and drug concentration

Association between venlafaxine last dose (end dose) at week 1 (Panel A) and week 12 (Panel B). The scatter plot represents the mean individual values; continuous line is the regression fit, and dashed lines are the 95% confidence interval for the latter.

Table 15: Correlation between dose and drug concentration

Dose	n	Concentration at week 1		Adjusted	
		Spearman (rs)	P-value	Beta (95% CI)	P-value
End dose at week 1	46	0.229	0.126	0.375 (0.803 to 4.601)	0.007*

Dose	n	Concentration at week 12		Adjusted	
		Spearman (rs)	P-value	Beta (95% CI)	P-value
End dose at week 12	42	0.758	<0.001*	0.752 (1.807 to 3.398)	<0.001*

Dose	Group	Concentration at week 12		Adjusted	
		Mean ± SD(n)	P-value	Beta (95% CI)	P-value
Dose Trajectory	Low	437.8 ± 217.6(20)	<0.001*	Reference	-
	High	705.5 ± 198.2(22)		0.512 (107.9 to 389.5)	0.001*

The association between end dose and drug concentration at week 1 and week 12 were assessed using Spearman correlation. The drug concentration at week 12 was compared to low and high MADRS trajectory using Student's t-test. Adjusted p-value was obtained after performing regression analysis controlling for covariates. Beta is the standardized regression coefficient. Statistical difference was established at *p<0.05.

3.3.5.2 Dose and Proposed Target Therapeutic Range

The relationship between venlafaxine end dose and dose trajectory with proposed therapeutic drug concentration range at week 1 and 12 is shown in Table 16-18. At week 1, there was no correlation between end dose and proposed therapeutic drug concentration range before

and after controlling for covariates. At week 12, the mean end dose was higher in patients that exceed the proposed therapeutic concentration range (259.8 ± 58.6 ng/ml) when compare to patients within the proposed target therapeutic concentration range (160.7 ± 30.9 ng/ml, p<0.001). In addition, patients in high dose trajectory group were ~15-fold more likely to achieve drug concentrations above the proposed therapeutic range at week 12 (p<0.001).

Table 16: Relationship between dose and with proposed target therapeutic concentration range at week 1

Therapeutic Range	End dose at week 1	Unadjusted P-value	Adjusted	
	Mean ± SD (n)		OR (95% CI)	P-value
Below Target Conc	75.0± 53.0 (2)	0.065	NA	NA
Within Target Conc	110.4 ± 12.5 (36)		Reference	NA
Above Target Conc	117.2 ± 13.3 (8)		1.059 (0.990 to 1.133)	0.097

The association between end dose at week 1 and proposed target therapeutic concentration range at week 1 was assessed using Kruskal-Wallis test. Adjusted p-value was obtained after performing regression analysis controlling for covariates. OR is the odd ratio and CI is the confidence interval. Reference group is the within proposed target concentration range. Since we have small samples size in the below target concentration range, we were not able to obtain OR and 95% CI for this group. NA= not applicable. Statistical difference was established at *p<0.05.

Table 17: Relationship between dose and with proposed target therapeutic concentration range at week 12

Therapeutic Range	End dose at week 12	Unadjusted P-value	Adjusted	
	Mean ± SD (n)		OR (95% CI)	P-value
Within Target Conc	160.7± 30.9 (14)	<0.001*	Reference	0.009*
Above Target Conc	259.8 ± 58.6 (28)		1.045 (1.011 to 1.079)	

The association between end dose at week 12 and proposed target therapeutic concentration range at week 12 was assessed using Mann-Whitne U test. Adjusted p-value was obtained after performing regression analysis controlling for covariates. OR is the odd ratio and CI is the confidence interval. Reference group is the within proposed target concentration range. Statistical difference was established at *p<0.05.

Table 18: Relationship between dose trajectory and with proposed target therapeutic concentration range at week 12

Therapeutic Range	Low dose trajectory	High dose trajectory	Unadjusted		Adjusted	
	n (%)	n (%)	OR (95% CI)	P-value	OR (95% CI)	P-value
Within Target Conc	12 (60.0%)	2 (9.1%)	15.0 (2.7-82.7)	<0.001*	Reference	0.020*
Above Target Conc	8 (40.0%)	20 (90.9%)			0.062 (0.006 to 0.644)	

The association between dose trajectory and proposed target therapeutic concentration range at week 12 was assessed using Chi-square test. Adjusted p-value was obtained after performing regression analysis controlling for covariates. OR is the odd ratio and CI is the confidence interval. Reference group is the within proposed target concentration range. Statistical difference was established at *p<0.05.

3.4 Discussion

This clinical study investigates the association between venlafaxine dose and drug concentration in depressed patients after 12 weeks of treatment with venlafaxine.

3.4.1 Description of Venlafaxine Dose Trajectory

In our study, the dosing protocols for venlafaxine was based on the recommendation obtained from the venlafaxine product label. Since the dose in our study is titrated over time based

mainly on the response and tolerability, we determined dose trajectory patterns over time in our patient population. We identified two distinct dose trajectory patterns which show a similar dosing regimen from 0-6 weeks followed by a sharp increase in dose in the "high" dose trajectory group from 6-12 weeks when compared to the "low" trajectory group. To the best of our knowledge, we are the first study to investigate the venlafaxine dose trajectory patterns over time in population.

3.4.2 Description of Proposed Therapeutic Drug Concentration Range

The proposed therapeutic range of venlafaxine drug concentration has been reported in previous studies.[143] This proposed therapeutic range is based largely on observational studies and expert opinion and the exposure-response relationship remains unclear.[144] Although this proposed target therapeutic concentration range has been recommended in the AGNP-TDM Expert Group Consensus Guidelines: Therapeutic Drug Monitoring in Psychiatry[143], TDM of venlafaxine is not currently recommended in the product label or the Practice Guideline for the Treatment of Patients With Major Depressive Disorder.[145] In our study, the majority of patients showed drug concentration within the proposed therapeutic range at week 1 and above the proposed therapeutic range at week 12, which is comparable to a previous report.[144]

3.4.3 Description of Drug Concentration

Drug concentration levels reported in our study were compared to other clinical studies. At the end of our study we observed a mean drug concentration of 578.02 ng/ml at a mean venlafaxine

dose of 221.8 mg/day. In studies by Sigurdsson et al. and Unterecker et al., depressed patients receiving venlafaxine at a mean dose of 199.1 to 207.8 mg/day showed a mean drug concentrations of 352.9 and 387.0 ng/ml, respectively.[142, 152] Although the dose at the end of our 12-week study was similar to final doses reported in these clinical studies, drug concentration is higher than previously published values.[142, 152] Since our patient population consists primarily of elderly MDD patients (mean age: 66.3±7.2 yrs, age range: 50-84 yrs), the higher drug concentrations in our study may be due to reduced drug clearance commonly observed in the elderly as previously suggested in other studies.[144] Other potential factors influencing the observed differences in concentration may include the analytical methods, protocol for dose titration, and duration of dosing. In addition, in our study we observed a high variability in the drug concentration at week 1 and week 12 even after adjusting for dose. Our results are similar to previous studies that reported venlafaxine exhibit high variability in drug concentration, dose-corrected drug concentration, and PK parameters.[29] Potential explanation for this large variability could be attributed to variation in drug absorption and/or distribution, genetic variability in metabolizing and eliminating venlafaxine, presence of renal and hepatic dysfunction, and drug-drug interactions.[153]

3.4.4 Description of Dose-Corrected Drug Concentration

The drug product label noted that venlafaxine exhibits linear PK over the dose range of 75-450 mg/day. Therefore, it is acceptable to dose-normalize drug concentration values in order to account for variable dosing regimens as reported in literature.[142, 144, 146, 152, 154] Dose-corrected drug concentration levels reported in our study were compared to other clinical studies. At the end of our study, we observed a mean dose-corrected drug concentration of 2.55 ng/ml/mg at a mean venlafaxine dose of 221.8 mg/day. Studies by Sigurdsson et al. and Unterecker 2014 et al. reported

that depressed patients received venlafaxine at a mean dose of 207.8 and 209.2 mg/day showed a mean dose-corrected drug concentrations of 1.79±1.09 and 1.48±0.685 ng/ml/mg, respectively.[146, 152]

However, after correcting for dose, our study reports higher drug concentration at week 12 compared to other studies. As previously mentioned, it is possible that the elderly patient population in our study demonstrate reduced drug clearance and thus higher drug concentration as suggested in other studies.[144] In fact, Sigurdsson et al have investigated the effect of age on dose-corrected drug concentration and demonstrated that elderly patients had higher dose-corrected drug concentration (2.4±1.2 ng/ml/mg) when compared to non-elderly patients (1.7±1.0 ng/ml/mg).[152] Other clinical studies have reported similar results.[142, 144, 154]

3.4.5 Relationship Between Dose and Drug Concentration

In our study, we observed a weak correlation between venlafaxine dose and drug concentration at an early time point (week 1) and a strong correlation at late time point (week 12). Previous clinical studies investigated the association between venlafaxine dose and drug concentration in TDM samples and reported either weak[142, 144] or moderate[146] correlations. A weak correlation between venlafaxine dose and drug concentration was generally observed in clinical studies with a shorter duration of dosing.[142, 144] Although it is expected to reach venlafaxine steady-state concentration after 3 days of multiple dosing[29], the dose in our study was titrated over time and therefore steady-state concentration levels may have changed over time. Collectively, these

data suggest that the dosing level and duration may impact the correlation between venlafaxine dose and drug concentration in depressed patients.

From a clinical perspective, our findings suggest that the venlafaxine dose may not be a clinically relevant predictor of drug concentration, and possibly clinical effect, in the early stages of treatment. Therefore, addition studies are needed to establish a target therapeutic concentration range based on safety and efficacy and to optimize the dosing regimen to achieve the target therapeutic range to possibly improve clinical response rates and/or reduce the time to clinical response.

3.4.6 Relationship Between Covariates and Dose/Drug Concentration

In our study, week 1 results showed no difference in the end dose between age groups, but patients above 65 years of age had significantly higher drug concentration and dose-corrected drug concentration. In addition, there was weak and moderate positive correlation between age and both drug concentration and dose-corrected drug concentration at week 1, respectively. At week 12, there was weak positive correlation between age and dose-corrected drug concentration and a trend for higher drug concentration in patients above 65 years of age. These results are in good agreement with a study by Hansen et al. which reported a similar venlafaxine dose among age groups, but higher venlafaxine drug concentration and dose-corrected drug concentration in patients ≥ 65 years of age.[144] In a similar fashion, studies by Sigurdsson et al. and Waade et al reported patients ≥ 65 years of age had higher mean drug concentration and dose-corrected concentration despite receiving a lower mean dose of venlafaxine.[152] Although Reis et al. did not

compare the venlafaxine dose among age groups, patients ≥ 65 years of age also had higher median drug concentration. [7]

The increase in drug concentration could possibly signify reduced renal elimination due to the physiologically reduced renal function in the elderly.[144] The venlafaxine product label notes that "The pharmacokinetics of venlafaxine and ODV are not substantially altered in the elderly. No dose adjustment is recommended for the elderly on the basis of age alone, although other clinical circumstances, some of which may be more common in the elderly, such as renal or hepatic impairment, may warrant a dose reduction".[29] In our study, we did not observe any significant differences in both venlafaxine dose and drug concentration in patients with renal and hepatic impairment when compared to patients without these comorbidities. However, only a few patients were diagnosed with renal and hepatic impairment thus limiting the power of the statistical analysis. Also, it is important to note that our study did not report any differences in venlafaxine dose and drug concentration between age groups at week 12. Taken together, these data would suggest that MDD patients over 65 years of age have higher drug concentration early after initiation of venlafaxine treatment possibly due to reduced drug clearance.

In addition, week 1 results showed no difference in the end dose or drug concentration between males and females. At week 12, males were more likely to be in the high dose trajectory group and achieved drug concentration above the target therapeutic range. Likewise, males showed a trend for higher drug concentration when compared to females. However, after adjusting for dose, we did not observe an association between dose-corrected concentration and gender. These findings suggest that increased drug concentration in males could be due to increased venlafaxine

dose. Consistent with our findings, Sigurdsson et al. reported that males received higher venlafaxine doses compared to females during treatment of late-life depression.[152] However, the study by Sigurdsson et al. also reported that males had lower drug concentration and dose-corrected concentration when compared to females, which is not consistent with our findings. In a similar fashion, Hansen et al. which reported an identical median and range of venlafaxine dose among males and females, but males had lower drug concentration and dose-corrected drug concentration when compared to females.[144]

The findings of Sigurdsson et al. suggest that males may have lower drug absorption, increased drug clearance, or other altered PK.[152] However, the Effexor XR (venlafaxine) drug label notes that "A population pharmacokinetic analysis of 404 venlafaxine-treated patients from two studies involving both b.i.d. and t.i.d. regimens showed that dose-normalized trough plasma levels of either venlafaxine or ODV were unaltered by age or gender differences. Dosage adjustment based on the age or gender of a patient is generally not necessary". Moreover, sex differences in venlafaxine dosing and concentration may be confounded by other factors including differences in weight or BMI.[155]

In addition, week 1 results showed no association between BMI and venlafaxine dose or drug concentration. Likewise, at week 12 results showed no association between BMI and venlafaxine dose, however we observed a weak negative correlation between BMI and drug concentration at week 12. Also, we found that patients who achieved drug concentration above the proposed target therapeutic range had lower BMI. These findings are similar to a previous study by Sigurdsson et al.[152] Venlafaxine is a lipophilic drug that distributed into body fat. Therefore,

increased body fat can lead to increased volume of distribution for venlafaxine, longer half-life, and lower drug concentration levels. [152] Therefore, dose adjustment should take into account the BMI levels. Collectively, these findings suggest that demographic and patients related factors such as age, sex and BMI should be taking into account during dose adjustment particularly in elderly patients taking venlafaxine treatment.

3.5 Conclusions

In summary, our study suggest that the dosing level and duration may impact the correlation between venlafaxine dose and drug concentration in depressed patients. In addition, our findings suggest that the venlafaxine dose may not be a clinically relevant predictor of drug concentration, and possibly clinical effect, in the early stages of treatment. Moreover, patient demographics and baseline clinical characteristics such as age, sex and BMI should be taken into account during venlafaxine dose adjustment. These findings suggest that the efficacy and safety of venlafaxine treatment of patients with MDD may be optimized through dose titration based on measurement of drug concentration possibly thought a TDM program.

4.0 Relationship Between Venlafaxine Dose, Drug Concentration, and Clinical Outcomes

4.1 Introduction

MDD is a devastating condition associated with increased risk of suicide and worsening of medical comorbidities which may be attributed to the lack of clinical predictors of response. Studies report that about 30% to 50% of patients with MDD fail to respond to adequate first-line treatment.[10] The conventional treatment of MDD requires dose titration based on clinical response and tolerability. Venlafaxine has a wider dosage range (75-375 mg daily) than most SSRIs and higher doses are associated with a greater incidence of adverse effects.[156] Despite dose titration, MDD patients on venlafaxine treatment have demonstrated variable response rates and treatment-resistant depression, and tolerability concerns.[10, 157, 158] The underlying factors contributing to the variability in clinical outcomes and tolerability issues in MDD patients on venlafaxine treatment are not well understood.[159] However, some studies report that demographic factors, such as age, sex, race, and BMI, weight, and lifestyle factors have been reported to influence the PK and/or PD of venlafaxine in depressed patients.[152]

The relationship between venlafaxine drug exposure and clinical response has not been fully investigated especially in elderly MDD patients, despite venlafaxine widespread use in this population. Thus, a better understanding of the impact of IIV in venlafaxine drug concentration on clinical outcomes may improve the treatment efficacy, reduce the treatment time required to achieve a clinically significant response, and potentially reduce the number and severity of adverse events associated with treatment. Given the clinical importance for identifying the sources of

variability in venlafaxine response in tailoring treatment, this goal of this chapter is to investigate the relationship between venlafaxine dose, drug concentration, and clinical outcomes in MDD patients and identify the impact of certain demographic and clinical factors on those relationships. We expect that concentration in plasma is the strongest predictor of clinical outcomes and that demographic and clinical factors affect the relationship between dose, concentration, and outcomes in MDD patients.

4.2 Materials and Methods

Detailed description of the study design and participants, dosing regimen, sampling and analytical methods are previously described in detail in chapter 3.

4.2.1 Depression Assessment

The depressive symptoms severity was assessed at baseline and then once a week for the first 2 weeks and then every 2 weeks using MADRS. This is a clinician-administered scale and consists of 10-questionnaire evaluating core symptoms of depression which include: 1) apparent sadness, 2) reported sadness, 3) inner tension, 4) reduced sleep, 5) reduced appetite, 6) concentration difficulties, 7) lassitude, 8) inability to feel, 9) pessimistic thoughts, and 10) suicidal thoughts. The total score ranges from 0 to 60 and higher scores indicate more severe symptoms. Also, MADRS is one of the most commonly used scales to evaluate the efficacy of antidepressant treatment in clinical trials and in clinical practice.

Three clinical outcomes are investigated in this study: clinical response, change in MADRS score, and MADRS trajectory. The clinical response is defined as a decrease of ≥50% from baseline MADRS score within the 12 weeks study duration. The change in MADRS score at week 12 is defined as the change in MADRS score from baseline to end of treatment. MADRS trajectory is a statistical approach to identify groups which share common patterns of change in MADRS scores over time.

4.2.2 Patient Demographics and Baseline Clinical Characteristics

Patient demographics assessed included age, sex (male/female), race (Caucasian/ African American), and level of education. Baseline clinical characteristics assessed included depression type (single/recurrent), comorbid medical burden as measured by cumulative illness rating scale-geriatric (CIRS-G), and baseline-MADRS.. Summary of the study design is shown in Figure 12.

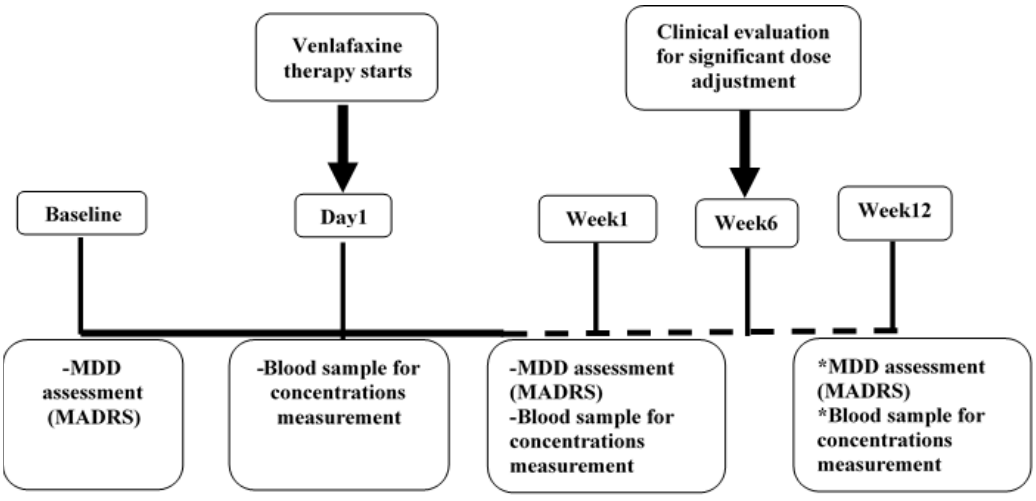


Figure 12: Chart summarizing the study design protocol

4.2.3 Statistical Analysis

Statistical analyses were performed using IBM SPSS statistical software version 24.0 (SPSS Inc., Chicago, IL, USA). In this study, we assessed the effect of venlafaxine dose and drug concentration on three clinical outcomes: clinical response, change in MADRS score and MADRS trajectory. This first outcome is clinical response, which is defined as ≥50% decrease from baseline MADRS score within the 12 weeks study duration. Clinical response is a binary variable (responder/non-responder) or categorical variable (early-responder, late-responder, non-responder). We defined early responder as subjects who responded halfway the study (at week 6) before significant dose adjustment, late-responder were defined as subjects who responded between week 6 and week 12 after significant dose adjustment, and non-responder are subject who did not respond to venlafaxine treatment within the 12 weeks study duration. The second outcome measure is the change in MADRS score from baseline to week 12. The negative change from baseline indicates a reduction (or improvement) in depressive symptoms. The third outcome is the MADRS trajectory pattern. This is a data-driven approach identifies groups which share common patterns of change in MADRS scores over time. The group-based trajectory analysis was performed using PROC TRAJ software package in SAS version 9.4. Unlike the clinical response, MADRS trajectory does not rely on a pre-specified response status. One advantage of MADRS trajectory over clinical response is that MADRS trajectory captures the variability in the response of the population over the study duration.[160]

In this study, the independent variables are venlafaxine end dose at week 1 and week 12, dose trajectory overtime, drug concentration at week 1 and week 12. Covariates included in the analysis are patient demographic factors, such as age, gender, race, and education, and baseline clinical characteristics, such as baseline MADRS scores, BMI, CIRS-G, and depression type. Age, education, weight, BMI, baseline-MADRS, and CIRS-G were evaluated as continuous variables. Gender, race, and depression type were evaluated as binary variables.

4.2.3.1 Test of association

Detailed description of test of association is previously described in Chapter 3.

4.2.3.2 Regression Analysis

We evaluated the effect of venlafaxine dose and drug concentration on clinical outcomes after adjusting for covariates using regression analysis. Logistic regression was used for our binary outcomes (responder/non-responder) and categorical outcomes (low/moderate/high MADRS trajectory). Linear regression was used for continuous outcomes (change in MADRS at week 12). For logistic regression analysis, we reported the odds ratio (OR) and 95% confidence interval (CI). For linear regression, we reported standardized regression coefficient and 95% CI.

(a) Linear Regression

We evaluated the relationship between venlafaxine dose, drug concentration and the change in MADRS score at week 12 after adjusting for covariates using linear regression analysis. In the linear regression analysis, we included covariates which demonstrated a trend for a significant association ($p<0.10$) with clinical outcomes. We also included certain covariates which have been previously reported to be significantly associated with clinical outcomes even if these variables were not statistically significant in our analyses.[149]

All potential predictors variables were then entered into a backward stepwise linear regression model to select the smallest subset of predictors variable that predict the change in MADRS score at week 12. Variables entered into the model included are age, gender, baseline-MADRS, BMI, CIRS-G, and depression type. Model entry and exit p-values were set at 0.10 and 0.20, respectively. These entries and exit p-values were chosen to prevent potentially important predictors from being excluded. While liberal p-values were chosen for selecting the final set of independent variables, we considered only those variables with $p<0.05$ as statistically significant. After defining our model, we checked whether the assumption for the models was met, we plotted a graph of the residuals vs. the predicted values to test the normality and constant variance of the residuals.

(b) Logistic Regression

A logistic regression was used to explore the relationship between predictors variables and achieving clinical response (2 groups: responder/non-responder). The same analysis was also used for MADRS trajectory (3 groups: mild, moderate, high). The same method described for covariate selection and model building for the linear regression were used to develop logistic regression.

Briefly, potential predictors were tested using univariate analysis. Potential predictors variables with a p-value ≤ 0.2 in the univariate analyses were included in a backward multivariate logistic regression procedure to evaluate which variables were independently associated with the likelihood of achieving clinical response.[150] The regression models were evaluated for goodness of fit using the Hosmer and Lemeshow test with receiver operating characteristic curve (ROC).[151]

(c) Cox Proportional Hazards Regression

We used multivariate cox proportional hazards regression models to test the association between the potential predictors and time to response. The estimated hazard ratio is the increase or decrease in risk, caused by the presence or absence of specific variable. In this analysis a HR<1.0 means a decrease in the "risk" of response and a therefore a longer duration of response. The same method described for covariate selection and model building for the linear regression were used to develop the cox proportional hazard.

4.3 Results

4.3.1 Description of the Clinical Outcomes (MADRS Trajectory, Change in MADRS, and Clinical Response)

4.3.1.1 MADRS Trajectory

The depressive symptoms severity profiles and trajectory patterns are shown in Figure 13. The trajectory model evaluating depressive scores show three groups of patients with significantly different depressive score profiles from weeks 0-12 after starting venlafaxine treatment ($p<0.001$). Patients in the "low" group (n=17, 34.5%) have relatively low mean depressive scores that decrease rapidly over time (week 0: 18.82±5.95, week 12: 3.57±4.43). Patients in the "Moderate" group (n=28, 48.1%) have relatively high mean depressive scores that decrease rapidly over time (week 0: 25.89±5.89, week 12: 10.72±6.39). Patients in the "high" group (n=9, 17.3%) have relatively high mean depressive score at week 1 that decrease modestly over time (week0: 29.67±5.65, week 12: 24.22±5.24)

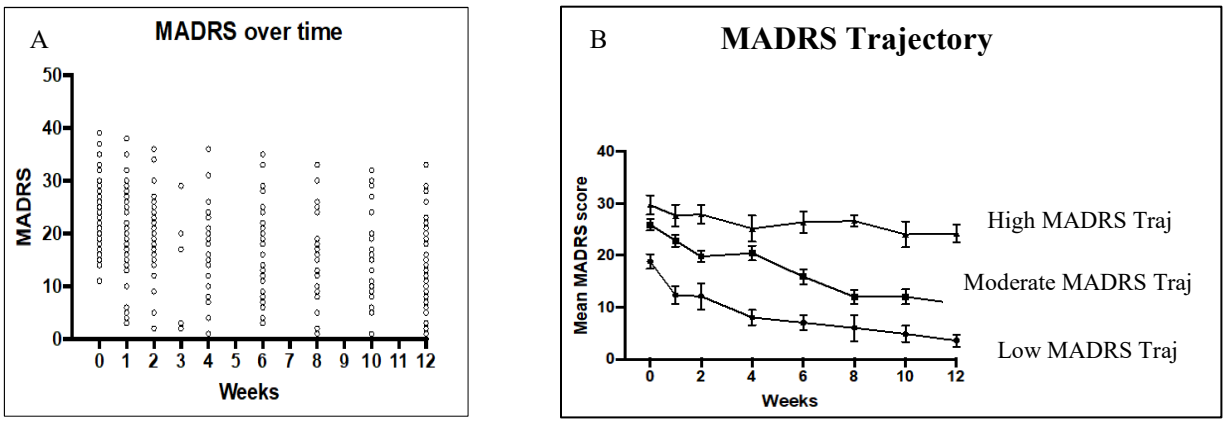


Figure 13: Trajectory patterns of depressive symptoms severity

Panel (A) shows raw population values of depressive symptoms severity as measured by MADRS score from 54 patients up to 12 weeks after starting venlafaxine treatment. Panel (B) shows the depressive symptoms severity as measured by MADRS versus time from starting venlafaxine treatment (weeks) in high {filled triangles, n=9 (17.3%)}, moderate {filled squares, n=28 (48.1%)} and low {filled circles, n=17 (34.5%)} groups as identified by trajectory analysis. MADRS data are presented as mean with 95% confidence interval.

4.3.1.2 Description of Change in MADRS Score at Week 12 (ΔMADRS scores)

Results showing the distribution of the change in MADRS scores at week 1 and week 12 is shown in Figure 14. The change in MADRS scores at week 1 and week 12 were normally distributed with mean value of -4.31±6.26 and -13.77±9.74, respectively.

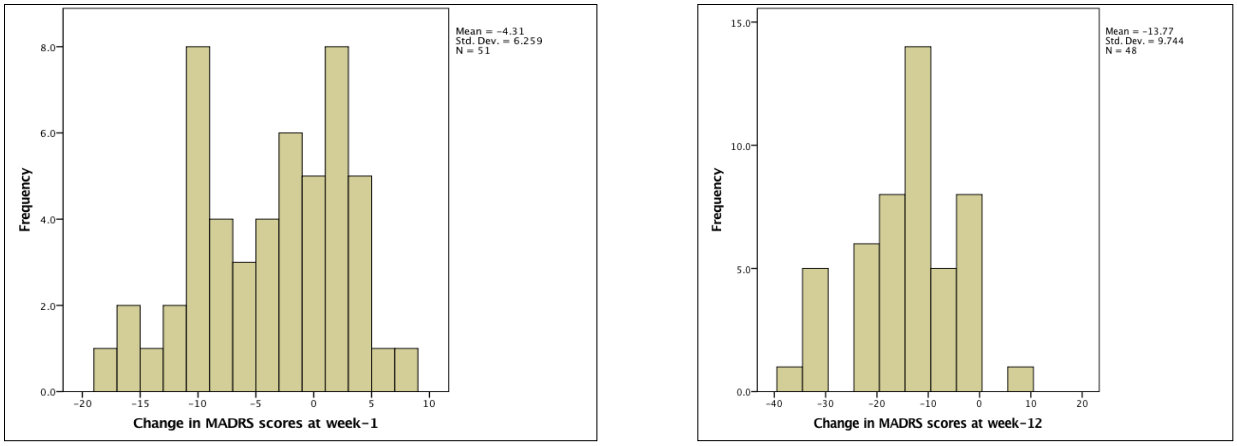


Figure 14: Histogram of change in MADRS score at week 1 and week 12

X-axis: The change in MADRS scores at week 1(Panel A) and week 12 (Panel B); y-axis: frequency of samples

4.3.1.3 Description of Clinical Response at Week 12

The percentage of patients achieved clinical response over 12-week study duration is shown in Figure 15. Approximately 54% and 46% of patients were classified as responders and non-responders, respectively. The percentage of patients achieved clinical response by week 6 (before significant dose adjustment) and between week 6 and week 12 (after significant dose adjustment) are shown in Figure 16. About 25% and 60% of patients responded before and after significant dose adjustment at week 6, respectively.

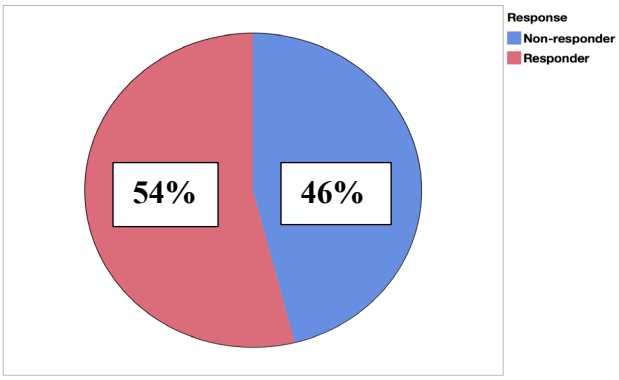


Figure 15: Pie chart for the parentage of patients achieved clinical response at the end of study

Clinical response is defined as a decrease of ≥50% from baseline MADRS score within the 12 weeks study duration. The blue, and red color represent the percentage of non-responder and responder, respectively.

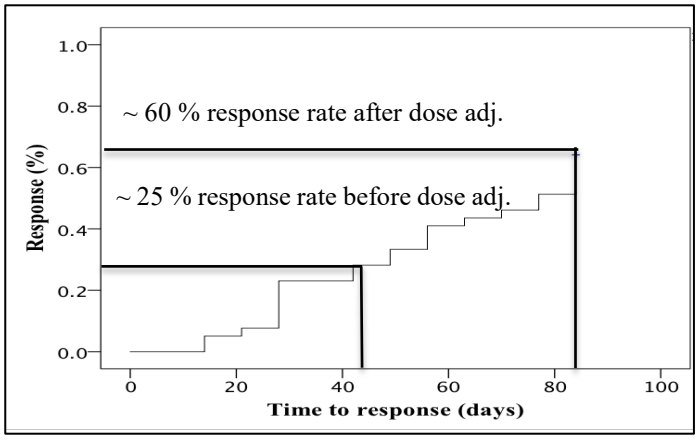


Figure 16: Time to clinical response

Kaplan-Meier survival curve for probability of response within 12-week study period.

4.3.1.4 Comparison of Outcomes

The mean change in MADRS score in the responder and non-responder groups is shown in Figure 17 and Table 19. The non-responder group had significantly lower change in MADRS score (worse outcome) ($p<0.001$) when compared to the responder group. The mean change in MADRS score in the MADRS trajectory group is shown in Table 20. Patients in the high MADRS trajectory group had significantly lower change in MADRS score (worse outcome) when compared to patients in the low MADRS trajectory group ($p=0.014$). Comparison of response in MADRS trajectory groups is shown in Table 21. Responders were more likely to be in the low MADRS trajectory group ($p<0.001$) when compared to non-responders.

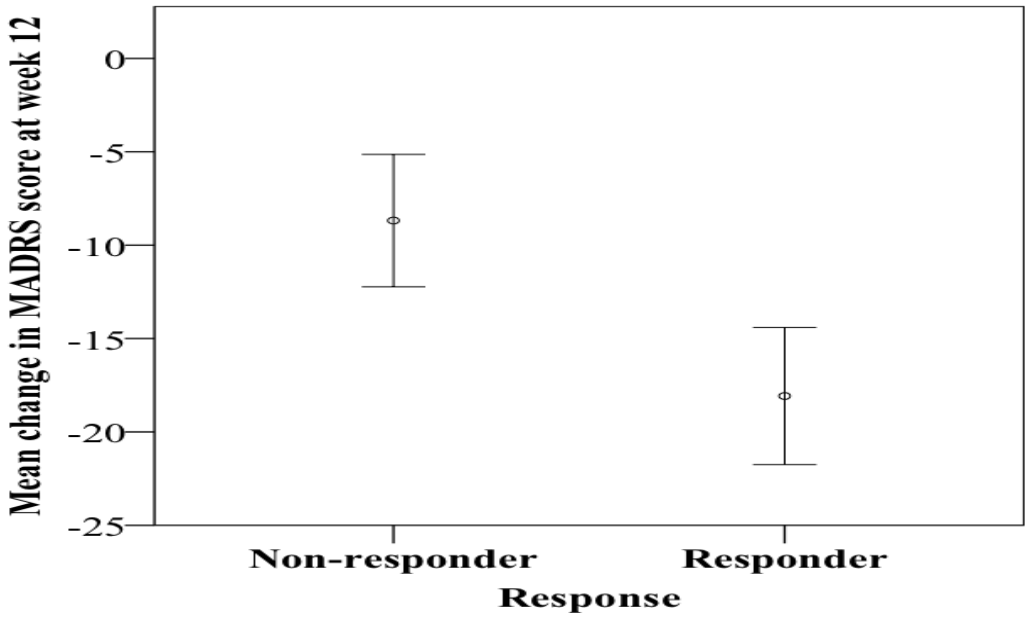


Figure 17: Change in MADRS score at week 12

This figure shows change in MADRS at week 12 in responder and non-responder. The change in MADRS at week 12 data are expressed as mean ± SEM. Data were compared using Student's t-test (2-tailed). Statistical difference was established at *p<0.05.

Table 19: Change in MADRS score at week 12 by response group

Response	Change in MADRS score at week 12	P-value
	Mean ± SD	
Non-Responder	-8.6 8 ± 7.99 (22)	<0.001*
Responder	-18.08 ± 9.08 (26)	

This table shows change in MADRS score at week 12 in responder and non-responder. The change in MADRS scores at week 12 data were expressed as mean ± SEM. Data were compared using Student's t-test (2-tailed). Statistical difference was established at *p<0.05.

Table 20: Change in MADRS score at week 12 by MADRS trajectory group

Outcome	MADRS Trajectory			P-value
	Low	Moderate	High	
	Mean ± SD (n)	Mean ± SD (n)	Mean ± SD (n)	
ΔMADRS score	-15.93±8.39 (14)	-15.56±10.15(25)	-5.44±6.15(9)^	0.014*

This table shows change in MADRS score at week 12 in low, moderate and high MADRS trajectory groups. The change in MADRS scores at week 12 data were expressed as mean ± SEM. Data were compared using ANOVA with Bonferroni's post-hoc test. Significant relationship from ANOVA post hoc analysis (*p<0.05) were noted when comparing low to high (^).

Table 21: MADRS trajectory by response group

Outcome	Group	MADRS Trajectory			P-value
		Low	Moderate	High	
		n (%)	n (%)	n (%)	
Response	Non-responder	0 (0)	14(60.9)	9 (39.1)	<0.001*
	Responder	14(51.9)	13(48.10	0(0)	

This table shows response in MADRS trajectory group. The response data were expressed as count (n) and percentage (%). Data were compared using Chi-square test. Statistical difference was established at *p<0.05.

4.3.2 Relationship Between Covariates and Clinical Outcomes

4.3.2.1 Covariates and MADRS Trajectory Patterns

The relationship between covariates and MADRS trajectory is shown in Table 22. Patients in the high MADRS trajectory group had higher baseline MADRS scores (29.67±5.6) when compared to patients in the low MADRS trajectory group (18.8±5.9, p< 0.001).

Table 22: Relationship Between Covariates and MADRS trajectory

Categorical Covariates					
Covariate	**Group**	**MADRS Trajectory**			**P-value**
		Low	**Moderate**	**High**	
		n (%)	**n (%)**	**n (%)**	
Age	≤ 65	7 (25.9)	14(51.9)	6(22.2)	0.46
	>65	10 (37)	14(51.9)	3(11.1)	
Gender	Male	6 (31.6)	7(36.8)	6 (31.6)	0.081
	Female	11 (31.4)	21(60)	3(8.6)	
Race	Caucasian	16 (35.6)	22(48.9)	7(15.6)	0.298
	African American	1 (11.1)	6(66.7)	2(22.2)	
Depression type	Singe	7 (36.8)	9(47.4)	3(15.8)	0.911
	Recurrent	10 (31.3)	16(50)	6(18.8)	
Continuous Covariates					
Covariate	**Group**	**MADRS Trajectory**			**P-value**
		Low	**Moderate**	**High**	
		Mean ± SD (n)	**Mean ± SD (n)**	**Mean ± SD (n)**	
Age (yr)	NA	65.94 ±8.8 (17)	67.29± 1.2(28)	63.89 ± 6.3 (9)	0.465
Age at first major depressive episode (yr)	NA	39.06 ±22.2 (16)	35.84 ±19.5 (25)	36.89 ±20.1 (9)	0.887
Education (yr)	NA	14.12 ±2.3 (17)	15.57 ±2.6 (28)	14.33 ±2.2 (9)	0.126
BMI	NA	30.35±7.4 (17)	30.263 ±5.7 (28)	29.172±5.2 (9)	0.884
CIRSG	NA	9.06±4.8 (17)	8.96±5 (28)	10.44±4.5 (9)	0.721
MMSE baseline	NA	29.31±1.1(16)	28.82±1.5(28)	28.50±1.1 (8)	0.313
MADRS baseline	NA	18.8±5.9 (17)^	25.89±5.9(28)	29.67±5.6(9)^	<0.001*

Categorical and continuous covariates were compared to low, moderate and high MADRS trajectory groups using Chi-square analysis and ANOVA with Bonferroni's post-hoc test, respectively. Significant relationships from ANOVA post hoc analysis ($p<0.05$) were noted when comparing low to high (^). BMI= Body Mass Index, CIRSG=Cumulative Illness Rating Scale for Geriatrics, MMSE= Mini Mental State Examination, MADRS= Montgomery and Asberg Depression Rating Scale, yr=year. Statistical difference was established at *$p<0.05$.

4.3.2.2 Relationship Between Covariates and Change MADRS scores at Week 12

The relationship between covariates and change MADRS score is shown in Table 23. Baseline MADRS score shows a weak negative correlation with change in MADRS score at week 12 ($r= -0.459$, $p=0.001$).

Table 23: Relationship between covariates and change in MADRS scores

Categorical Covariates			
Covariate	**Group**	**Change in MADRS week 12**	
Age	≤ 65	-13 ± 10.855 (24)	0.589
	>65	-14.54 ± 8.658 (24)	
Gender	Male	-11.4 ± 11.038 (15)	0.26
	Female	-14.85 ± 9.073 (33)	
Race	Caucasian	-13.05 ± 9.567 (39)	0.292
	African American	-16.89 ± 10.470 (9)	
Depression type	Singe	-14.35 ± 9.591 (17)	0.844
	Recurrent	-13.75 ± 10.131 (28)	
Continuous Covariates			
Covariate	**n**	**Change in MADRS week 12**	
		Pearson (r)	**P-value**
Age (yr)	48	0.034	0.82
Age at first major depressive episode (yr)	44	0.033	0.831
Education (yr)	48	0.064	0.663
BMI	48	-0.095	0.522
CIRSG	48	0.046	0.758
MMSE baseline	47	0.086	0.563
MADRS baseline	48	-0.459	0.001*

The association between the change in MADRS score at week 12 and categorical and continuous covariates were assessed using Student's t-test and Pearson correlation, respectively. BMI= Body Mass Index, CIRSG=Cumulative Illness Rating Scale for Geriatrics, MMSE= Mini Mental State Examination, MADRS= Montgomery and Asberg Depression Rating Scale, yr=year. Statistical difference was established at *p<0.05.

4.3.2.3 Covariates and Clinical Response

The relationship between covariates and clinical response is shown in Table 24. The non-responder group had lower baseline MADRS scores (22.7±7.5) when compared to the responder group (27.39 ± 5.3, p=0.016).

Table 24: Relationship between covariates and clinical response

Categorical Covariates					
Covariate	**Group**	**Non-responders**	**Responders**	**OR (95% CI)**	**P-value**
		N (%)	**N (%)**		
Age	≤ 65	14 (53.8%)	12 (46.2%)	1.944 (0.628-6.021)	0.272
	> 65	9 (37.5%)	15 (62.5%)		
Gender	Male	10 (58.8)	7 (41.2)	2.198 (0.667- 7.238)	0.192
	Female	13 (39.4)	20 (60.6)		
Race	Caucasian	18 (43.9)	23 (56.1)	0.626 (0.147-2.675)	0.715
	African American	5 (55.6)	4 (44.4)		
Depression type	Single	9 (50)	9 (50)	1.231 (0.379-4.0)	0.771
	Recurrent	13 (44.8)	16 (55.4)		
Continuous Covariates					
Covariate	**Group**	**Non-responders**	**Responders**	**OR (95% CI)**	**P-value**
		Mean ± SD (n)	**Mean ± SD (n)**		
Age (yr)	NA	65.35 ± 6.4 (23)	66.48± 6.9 (27)	NA	0.552
Age at first major depressive episode (yr)	NA	34 ± 19.1 (22)	38.92 ± 21.7 (24)	NA	0.421
Education (yr)	NA	15.13 ± 2.6 (23)	14.48 ± 2.5 (27)	NA	0.369
BMI	NA	29.871 ± 4.9 (23)	30.89 ± 7 (27)	NA	0.561
CIRSG	NA	9.35 ± 5.2 (23)	9.44 ± 4.2 (27)	NA	0.942
MMSE baseline	NA	28.91 ± 1(22)	28.96 ± 1.5 (27)	NA	0.89
MADRS baseline	NA	27.39 ± 5.3 (23)	22.7 ± 7.5 (27)	NA	0.016*

Categorical and continuous covariates were compared to responder and non-responder group using Chi-square analysis and Student's t-test, respectively. BMI= Body Mass Index, CIRSG=Cumulative Illness Rating Scale for Geriatrics, MMSE= Mini Mental State Examination, MADRS= Montgomery and Asberg Depression Rating Scale, yr=year. Statistical difference established at *p<0.05.

4.3.3 Baseline MADRS scores

The cumulative response rate during venlafaxine treatment grouped by baseline MADRS is shown in Figure 18. The cumulative response in patients above or below median split of baseline-MADRS shows that patients below median baseline-MADRS had a greater cumulative response (83.3%) over 12-weeks compared to those with above median baseline-MADRS (47.6%, p=0.015). This relationship did not change after controlling for clinical covariates.

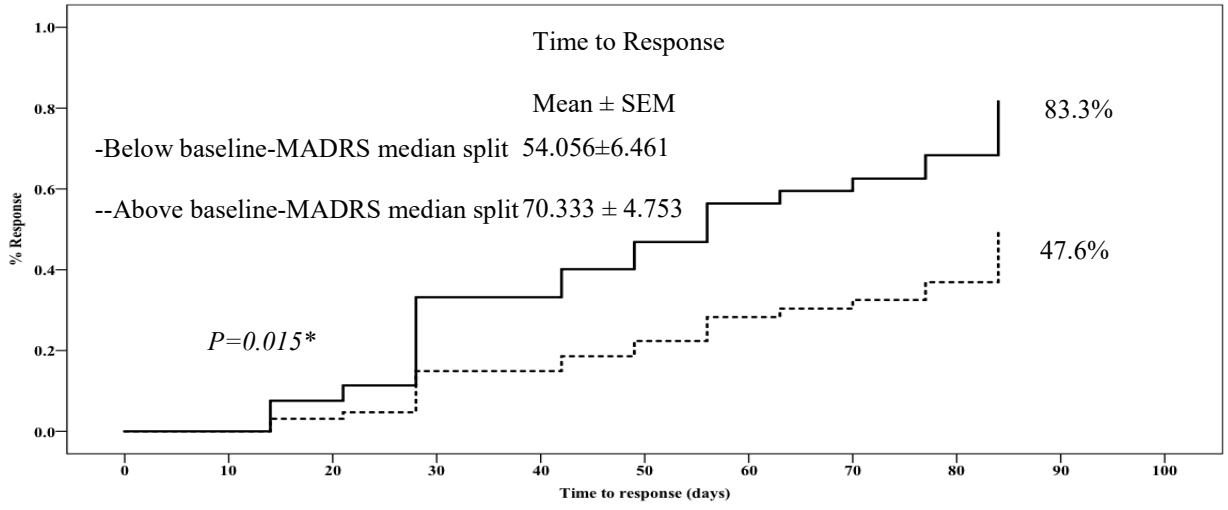


Figure 18: Cumulative response rate during venlafaxine 12-week treatment by baseline MADRS group

Cumulative response rate during venlafaxine 12-week treatment is compared in patients below baseline-MADRS (51.9%) and above baseline-MADRS (48.1%) groups using Kaplan-Meier log rank analysis (n=54). Statistical difference established at *p<0.05.

4.3.4 Relationship Between Venlafaxine Dose and Clinical Outcomes

4.3.4.1 Relationship Between Venlafaxine Dose and MADRS Trajectory

The relationship between MADRS trajectory groups and both end dose and dose trajectory groups shown in Figure 19 and Tables 25-27. At week 1, we did not observe an association between end dose at week 1 and MADRS trajectory. At week 12, we found that patients in the low MADRS trajectory group had lower end dose (179.5 ± 53.4 mg/day) when compared to patients in the high MADRS trajectory group (276.6 ± 52.8 mg/day, p=0.004). This relationship did not change after controlling for covariates. Moreover, patients in the low MADRS trajectory where more likely to fall in the low dose trajectory when compared to patients in the high MADRS trajectory (p=0.021) before and after controlling for covariates.

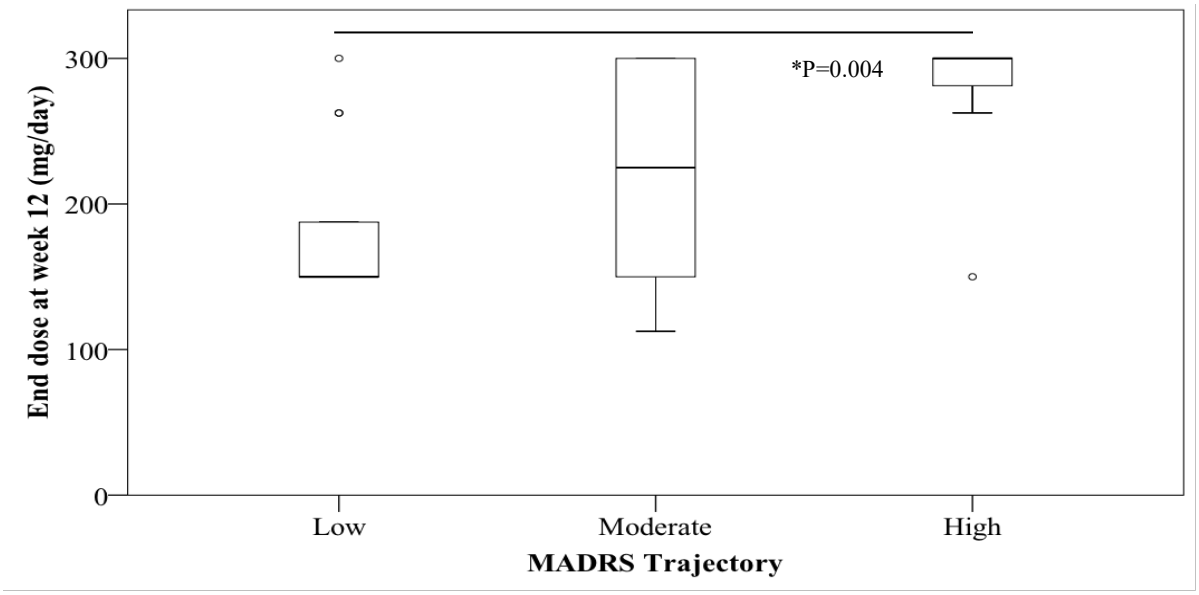


Figure 19: End dose in MADRS trajectory group

Box plot for end dose in the MADRS trajectory groups (Low, Moderate, High). The end dose was presented as box plot with median, minimum and maximum values and lower and upper quartiles. Outliers (circle) are cases with values outside the interquartile range.

Table 25: End dose at week 1 in MADRS trajectory groups

MADRS Trajectory	End dose at week 1		Unadjusted P-value	Adjusted	
	Mean ± SD (n)	Median		OR (95%CI)	P-value
Overall					<0.001*
Low	110 ± 9.68 (15)	112.5	0.854	1.037 (0.971 to 1.107)	0.282
Moderate	111.06 ± 16.7 (26)	112.5		1.033 (0.985 to 1.084)	0.181
High	104.167 ± 25 (9)	112.5		Reference	-

The end dose at week 1 was compared to low, moderate and high MADRS trajectory groups using Kruskal Wallis test. Regression analysis was performed after adjusting for covariates. Reference group is high MADRS trajectory. Adjusted odd ratio (OR) and 95% confidence interval (CI) were shown in table. Statistical difference was established at *p<0.05.

Table 26: End dose at week 12 in MADRS trajectory groups

MADRS Trajectory	End dose at week 12		Unadjusted P-value	Adjusted	
	Mean ± SD (n)	Median		OR (95%CI)	P-value
Overall					<0.001*
Low	179.464 ± 53.396 (14)^	150	0.006*	0.978 (0.957 to 0.999)	0.04*
Moderate	228.0 ± 70.089 (25)	225		0.988 (0.970 to 1.007)	0.221
High	276.563 ± 52.796 (8)	300		Reference	-

The end dose at week 12 was compared to low, moderate and high MADRS trajectory groups using Kruskal Wallis test. Bonferroni's post hoc analysis was performed comparing the high MADRS trajectory (reference group) with low MADRS trajectory and moderate MADRS trajectory and statistical difference was established at ^p<0.05. Regression analysis was performed after adjusting for covariates. Statistical difference was established at *p<0.05. Odd ratio (OR); 95% confidence interval (95%CI).

Table 27: Dose trajectory in MADRS trajectory groups

MADRS Trajectory	Dose Trajectory		Unadjusted P-value	Adjusted	
	Low	High			
	n (%)	n (%)		OR (95%CI)	P-value
Overall					<0.001*
Low	11 (47.8)	3 (12)	0.033*	0.025 (0.001 to 0.575)	0.021*
Moderate	11 (47.8)	14 (56)		0.219 (0.018 to 2.692)	0.235
High	1(4.3)	8 (32)		Reference	-

Overall association between MADRS trajectory and dose trajectory was compared using Chi-square test. Regression analysis was performed after adjusting for covariates. Reference group is the high MADRS trajectory. Statistical difference was established at *p<0.05. Odd ratio (OR); 95% confidence interval (95%CI).

4.3.4.2 Relationship Between Venlafaxine Dose and Change in MADRS

The relationship between end dose and dose trajectory with change in MADRS at week 1 and week 12 are shown in Figure 20 and Tables 28 to 30. We did not observe an association between end dose and dose trajectory with change MADRS at week 12. However, patients in the high dose trajectory group had a lower change in MADRS score at week 12 (worse outcome) when compared to patients in the low dose trajectory group after controlling for covariates.

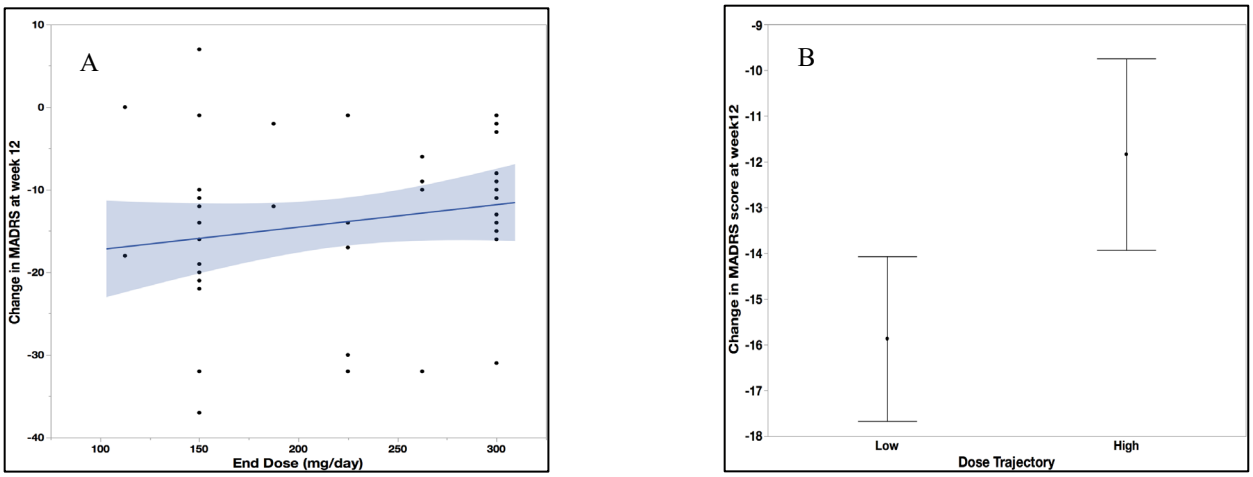


Figure 20: Association between dose and change in MADRS at week 12

Panel A shows the association between end dose and change in MADRS at week 12. Scatter plot represents the mean individual values; continuous line is the regression fit, and shaded area represent the 95% confidence interval for the fitted line. Panel B shows the change in MADRS in low dose trajectory group versus high dose trajectory group. The change in MADRS at week 12 is compared to low and high dose trajectory using Student's t-test. The change in MADRS at week 12 data is presented as mean (± SEM). Statistical difference was established at *p<0.05.

Table 28: End dose and change in MADRS at week 12

Outcome	n	End dose at week 12	Unadjsuted p-value	Adjusted	
		Spearman correlation (rs)		Beta (95% CI)	P-value
ΔMADRS at week 12	46	0.265	0.075	0.273 (-0.001 to 0.079)	0.056

The association between end dose at week 12 and change in MADRS score at week 12 was assessed using Spearman correlation. Adjusted p-value was obtained after performing regression analysis controlling for covariates. Beta is the standardized regression coefficient. Statistical difference was established at *p<0.05.

Table 29: End dose at week 1 and change in MADRS at week 1

Outcome		End dose at week 1	Unadjsuted p-value	Adjusted	
	n	Spearmsn correlation (rs)		Beta (95% CI)	P-value
Δ MADRS at week 1	46	-0.033	0.821	-0.133 (-0.169 to 0.063)	0.361

The association between end dose at week 1 and change in MADRS score at week 1 was assessed using Spearman correlation. Adjusted p-value was obtained after performing regression analysis controlling for covariates. Beta is the standardized regression coefficient. Statistical difference was established at *p<0.05.

Outcome	End dose at week 1		Unadjusted p-value	Adjusted	
	Mean ± SD (n)	Median		OR (95% CI)	P-value
Non-Responder	110.9 ± 17.79 (23)	112.5	0.297	Reference	0.917
Responder	108.0 ± 16.49 (25)	112.5		0.998 (0.961 to 1.037)	

The association between end dose at week 1 and change in MADRS score at week 1 was assessed using Spearman correlation. Adjusted p-value was obtained after performing regression analysis controlling for covariates. Beta is the standardized regression coefficient. Statistical difference was established at *p<0.05.

Table 30: Dose trajectory and change in MADRS at week 12

DoseTrajectory	ΔMADRS at week 12	Unadjusted P-value	Adjusted	
	Mean ± SD (n)		Beta (95%CI)	P-value
Overall				<0.001*
Low	-15.87 ± 8.636 (23)	0.154	Reference	-
High	-11.84 ± 10.463 (25)		0.403 (2.572 to 13.121)	0.004*

The change in MADRS score at week 12 was compared to low and high dose trajectory using Student's t-test. Regression analysis was performed after controlling for covariates. Beta is the standardized regression coefficient. Statistical difference was established at *p<0.05.

4.3.4.3 Relationship Between Venlafaxine Dose and Clinical Response

The relationship between the clinical response group and both end dose and dose trajectory group is shown in Figure 21 and Tables 31 to 33. The non-responder group had higher end dose at week 12 (255.3 ± 65.6 mg/day)when compared to the responder group (196.5 ± 62.4 mg/day, p=0.003). This relationship did not change after controlling for covariates. In addition, the non-responders were more likely to fall in the high dose trajectory group when compared to the responders (p=0.008). This relationship did not change after controlling for covariates.

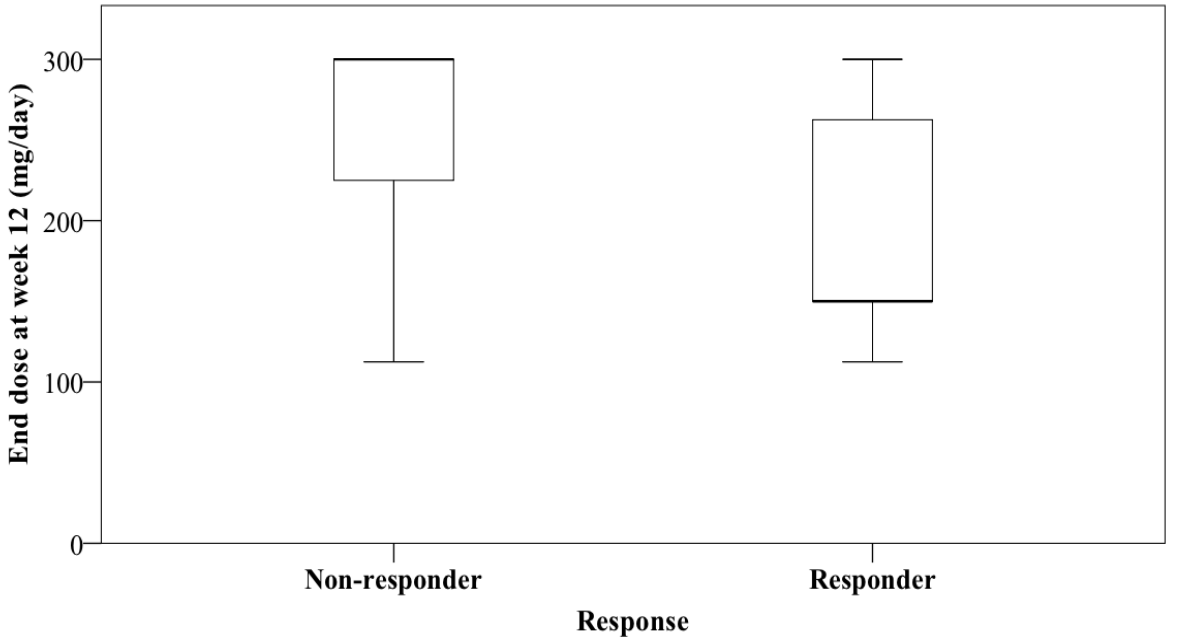


Figure 21: End dose in clinical response groups

Box plot for end dose in the clinical response groups (non-responder, responder). The end dose at week 12 is presented as box plot with median, minimum and maximum values and lower and upper quartiles.

Table 31: End dose at week 1 in response groups

Outcome	End dose at week 1		Unadjusted p-value	Adjusted	
	Mean ± SD (n)	Median		OR (95% CI)	P-value
Non-Responder	110.9 ± 17.79 (23)	112.5	0.297	Reference	0.917
Responder	108.0 ± 16.49 (25)	112.5		0.998 (0.961 to 1.037)	

The end dose at week 1 was compared to non-responder and responder using Mann-Whitney test. Regression analysis was performed after adjusting for covariates. Reference group is the non-responder. Statistical difference was established at *p<0.05. Odd ratio (OR); 95% confidence interval (95%CI).

Table 32: End dose at week 12 in response groups

Outcome	End dose at week 12		Unadjusted p-value	Adjusted	
	Mean ± SD (n)	Median		OR (95% CI)	P-value
Non-Responder	255.4 ± 65.62 (21)	300	0.003*	Reference	0.014*
Responder	196.5 ± 62.45 (25)	150		0.988 (0.978 to 0.997)	

The end dose at week 12 was compared to non-responder and responder using Mann-Whitney test. Regression analysis was performed after adjusting for covariates. Reference group is the non-responder. Statistical difference was established at *p<0.05. Odd ratio (OR); 95% confidence interval (95%CI).

Table 33: Dose trajectory in response groups

Outcome	Dose Trajectory		Unadjusted p-value	Adjusted	
	Low	High		OR (95% CI)	P-value
	n (%)	n (%)			
Non-Responders	6(27.3)	16(72.7)	0.008*	Reference	0.022*
Responders	17(65.4)	9(34.6)		4.964 (1.262 to 19.519)	

The dose trajectory was compared to non-responder and responder using Chi-square test. Regression analysis was performed after adjusting for covariates. Reference group is the non-responder. Statistical difference was established at *p<0.05. Odd ratio (OR); 95% confidence interval (95%CI).

4.3.5 Relationship Between Drug Concentration and Clinical Outcomes

4.3.5.1 Drug concentration and MADRS Trajectory

The relationship between MADRS trajectory groups and drug concentration at week 1 and 12 is shown in Figure 22 and Table 34 and Table 35. Patients in the low MADRS trajectory group had higher drug concentration at week 1 (365.2 ± 130.8 ng/ml) when compared to patients in the high MADRS trajectory group (225.8 ±179.5 ng/ml, p=0.039) after controlling for covariates. In addition, patients in the low MADRS trajectory group had lower drug concentration at week 12 (477.5 ± 252.4 ng/ml) when compared to patients in the high MADRS trajectory group (772.83 ± 174.44 ng/ml, p=0.035) after controlling for covariates.

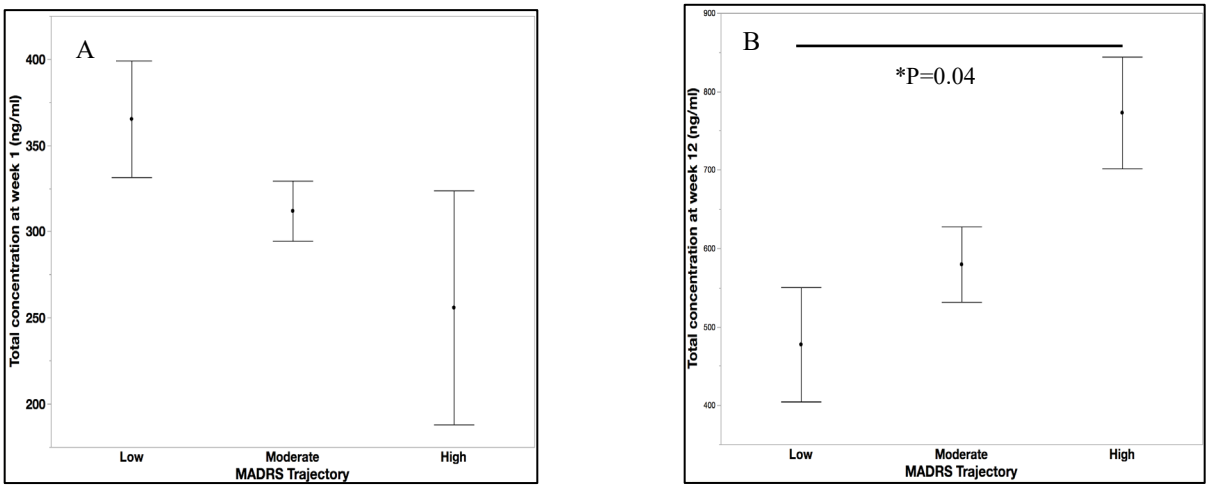


Figure 22: Drug concentration and MADRS trajectory relationship

The association between MADRS trajectory and drug concentration at week 1 (Panel A) and week 12 (Panel B). Drug concentration was compared in the low, moderate and high MADRS trajectory groups using ANOVA

with Bonferroni post hoc test. Drug concentration data were presented as mean ± SEM. Statistical difference was established at *p<0.05.

Table 34: Drug concentration at week 1 and MADRS trajectory

MADRS Trajectory	Concentration at week 1	Unadjusted P-value	Adjusted	
	Mean ± SD (n)		OR (95%CI)	P-value
Overall				<0.001
Low	365.2± 130.78 (15)	0.125	1.016 (1.001 to 1.031)	0.039*
Moderate	311.92 ± 85.4 (24)		1.003 (0.999 to 1.013)	0.455
High	255.8±179.55(7)		Reference	-

Overall association between MADRS trajectory and drug concentration at week 1 was compared using ANOVA and statistical difference was established at *p<0.05. Bonferroni's post hoc analysis was performed comparing the high MADRS trajectory (reference group) with low MADRS trajectory and moderate MADRS trajectory. Statistical difference was established at *p<0.05. Regression was performed adjusting for clinical covariates.

Table 35: Drug concentration at week 12 and MADRS trajectory

MADRS Trajectory	Concentration at week 12	Unadjusted P-value	Adjusted	
	Mean ± SD (n)		OR (95%CI)	P-value
Overall				<0.001*
Low	477.5±252.4(12)^	0.052	0.986 (0.973 to 0.999)	0.035*
Moderate	579.6±235.245(24)		0.990 (0.979 to 1.002)	0.097
High	772.83±174.44(6)^		Reference	-

Overall association between MADRS trajectory and drug concentration at week 12 was compared using ANOVA and statistical difference was established at *p<0.05. Bonferroni's post hoc analysis was performed comparing the high MADRS trajectory (reference group) with low MADRS trajectory and moderate MADRS trajectory. Statistical difference was established at ^p<0.05. Regression was performed adjusting for clinical covariates.

4.3.5.2 Relationship Between Drug Concentration and Change in MADRS

The relationship between drug concentration at week 1 and 12 and change MADRS at week 12 is shown in Figure 23 and Table 36. At week 1, we did not observe an association between drug concentration and change in MADRS scores at the end of study even after adjusting for covariates. However, we observed a weak positive relationship between drug concentration at week 12 and change in MADRS (r=0.307, p=0.043). This relationship did not change after adjusting for covariates.

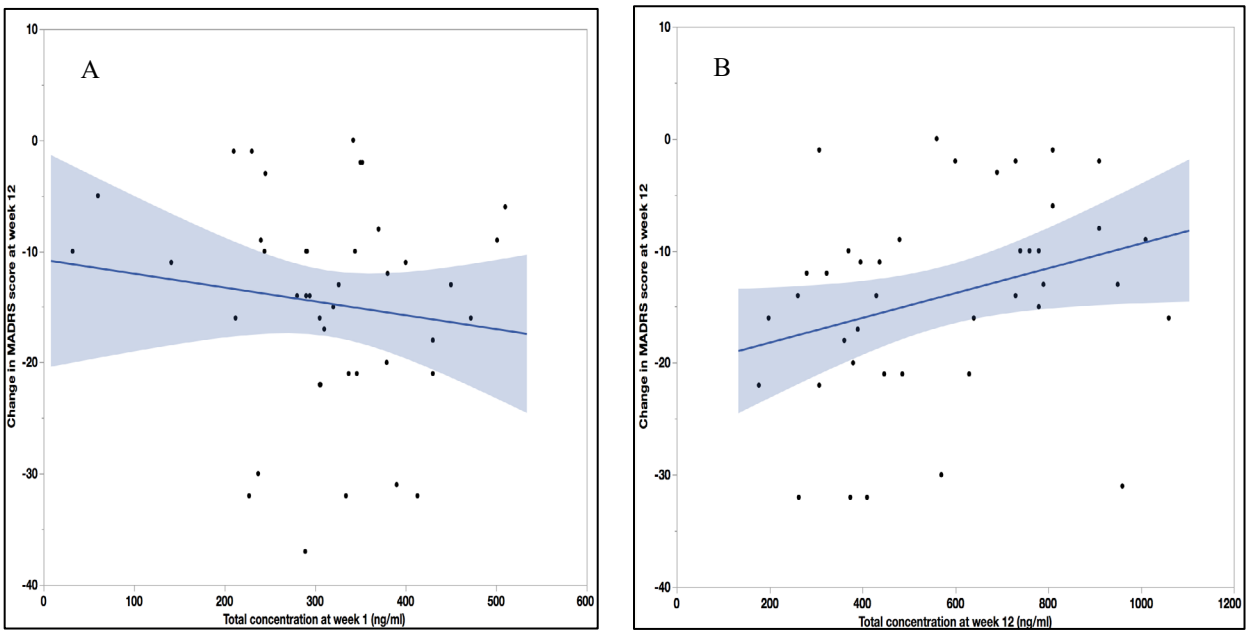


Figure 23: Drug concentration and change in MADRS at week 12

The association between change in MADRS at week 12 and concentration at week 1 (Panel A) and week 12 (Panel B). Scatter plot represents the mean individual values; continuous line is the regression fit, and shaded area represent the 95% confidence interval for the fitted line.

Table 36: Drug concentration and change in MADRS at week 12

Outcome	Concentration at week 1	Unadjsuted p-value	Adjusted	
	Pearson (r)		Beta (95% CI)	P-value
∆ MADRS at week 1	-0.134	0.398	-0.192 (-0.044 to 0.008)	0.177

Outcome	Concentration at week 12	Unadjsuted p-value	Adjusted	
	Pearson (r)		Beta (95% CI)	P-value
∆ MADRS at week 12	0.307	0.043*	0.274 (0.00015 to 0.02)	0.047*

The association between concentration and change in MADRS score was assessed using Spearman correlation. Adjusted p-value was obtained after performing regression analysis controlling for covariates. Beta is the standardized regression coefficient. Statistical difference was established at *p<0.05.

4.3.5.3 Relationship Between Drug Concentration and Clinical Response

The relationship between drug concentration at week 1 and week 12 and clinical response are shown in Figure 24 and Table 37. At week 1, we did not observe an association between drug concentration and clinical response. However, at week 12, non-responders had higher drug concentration at week 12 (688.53 ± 250.79 ng/ml) when compared to responders (486.7 ± 204.4 ng/ml, p=0.006) after controlling for covariates.

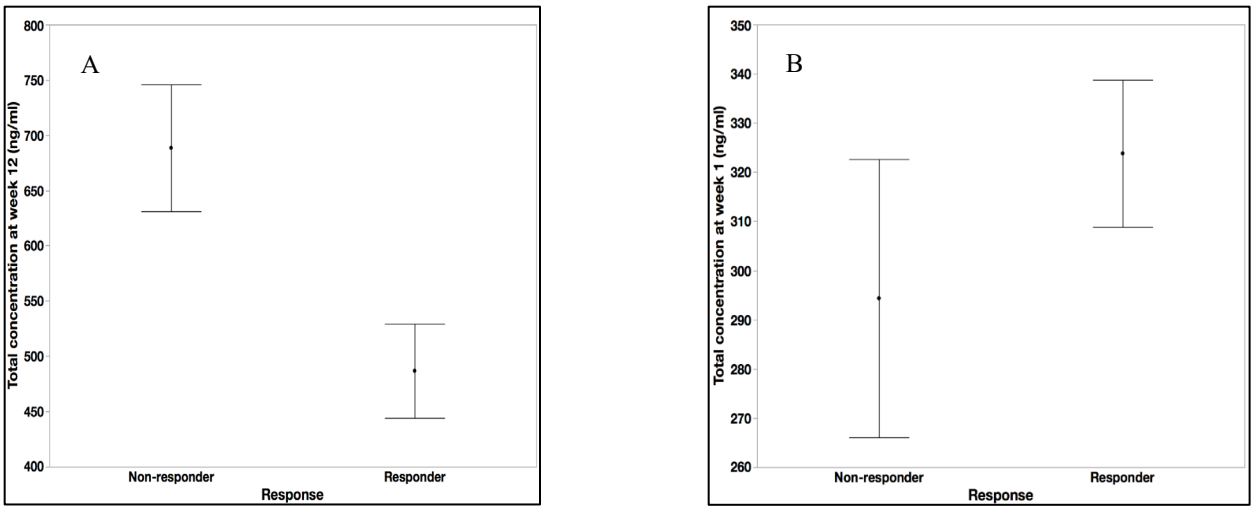


Figure 24: Drug concentration and clinical response group

The drug concentration at week 1 and 12 were represented as mean standard error of the mean (SEM). Statistical difference was established at *p<0.05.

The association between clinical response and drug concentration at week 1 (Panel A) and week 12 (Panel B). Drug concentration was compared responder and non-responder using Student's t-test. Drug concentration data were presented as mean ± SEM. Statistical difference was established at *p<0.05.

Table 37: Drug concentration and clinical response group

Outcome	Concentration at week 1	Unadjusted p-value	Adjusted	
	Mean ± SD (n)		OR (95% CI)	P-value
Non-Responders	294.3± 126.4 (20)	0.34	Reference	0.56
Responders	323.8 ± 73.2 (24)		1.002 (0.995 to 1.008)	

Outcome	Concentration at week 12	Unadjusted p-value	Adjusted	
	Mean ± SD (n)		OR (95% CI)	P-value
Non-Responders	688.53 ± 250.79 (19)	0.006*	Reference	0.029*
Responders	486.74 ± 204.38 (23)		0.996 (0.933 to 1.00)	

Drug concentration at week 1 and week 12 was compared in non-responder and responder using Student's t-test. Regression analysis was performed after controlling for covariates. Statistical difference was established at *p<0.05. Odd ratio (OR); 95% confidence interval (95%CI).

4.3.6 Analysis in Responder and Non-responder

We also investigated the association between dose, drug concentration, and clinical outcomes in a subset of responder and non-responder groups as shown in Table 38. We did not find any significant association between end dose and change in MADRS scores at week 12 in either group even after controlling for covariates. However, we found trend for positive weak association between drug concentration at week12 and change in MADRS score at week 12 in the responder group only (r=0.399, p=0.059). However, this relationship did not hold after controlling for covariates.

Table 38: Association between dose, drug concentration and change in MADRS by response

Non-responder					
Potential Predictors	**n**	**Δ MADRS at week 12**	**Unadjusted p-value**	**Adjusted**	
		Spearman (rs)		**Beta (95% CI)**	**P-value**
End dose	21	-0.31	0.171	-0.109 (-0.076 to 0.048)	0.651
Total concentration at week 1	19	-0.155	0.525	-0.263 (-0.042 to 0.012)	0.247
Total concentration at week 12	19	0.075	0.759	0.017 (-0.013 to 0.014)	0.939
Responder					
Potential Predictors	**n**	**Δ MADRS at week 12**	**Unadjusted p-value**	**Adjusted**	
		Spearman (rs)		**Beta (95% CI)**	**P-value**
End dose	25	0.33	0.107	0.2 (0.002 to 0.058)	0.037*
Total concentration at week 1	23	0.039	0.85	-0.168 (-0.047 to 0.005)	0.103
Total concentration at week 12	23	0.399	0.056	0.156 (-0.003 to 0.017)	0.177

The association between dose/drug concentration and change in MADRS score in responder and non-responder was assessed using Spearman correlation. Adjusted p-value was obtained after performing regression analysis controlling for covariates. Beta is the standardized regression coefficient. Statistical difference was established at *p<0.05.

4.4 Discussion

To the best of our knowledge, this is the first clinical study to compare the impact of venlafaxine dose and drug concentration on clinical outcomes, as measured by clinical response, MADRS trajectory and change in MADRS score, over a 12-week period in patients with MDD. The results of this clinical study revealed that venlafaxine dose and drug concentration predicted clinical outcomes at week 12 to a similar extent. Our findings did not demonstrate that drug concentration is superior than dose in predicting clinical outcomes in patients with MDD. Further clinical studies with larger sample sizes are needed to fully investigate the relationship between venlafaxine dose/drug concentration and clinical outcomes in patients with MDD.

4.4.1 Description of Clinical Outcomes

The clinical outcomes values in our study were compared to values reported in previous clinical studies. In our study, we observed a mean change of MADRS score -13.8 at week 12. Previous clinical studies by Thase et al and Cunningham et al. showed that patients on venlafaxine treatment had mean change in MADRS scores ranged from -12.7 to -13.2 at the end of the study.[161, 162] These findings are similar to our reported values for change in MADRS score at week 12. However, other studies have reported higher mean change in MADRS scores range from -17.5 to -19.3 during venlafaxine treatmen.[163, 164] In addition, previous clinical studies have reported a various response rate to venlafaxine treatment ranging from 39% to 75%.[164-166] This is consistent with our findings where we reported a response rate of 54% at the end of study. Possible explanation for these observed differences in the clinical outcomes could be related to higher

baseline MADRS score, different dosing protocols, longer duration of venlafaxine treatment, different measures of outcomes such as Hamilton Depression Rating Scale (HAM-D).

4.4.2 Relationship Between Venlafaxine Dose and Clinical Outcomes

Our study showed lack of correlation between dose and change in MADRS scores at the end of the study. There are few clinical studies that investigated the relationship between venlafaxine dose and clinical outcomes in depressed patients. A clinical study by Charlier et al have reported no correlation between venlafaxine dose and MADRS score in depressed patients.[167] Although we used a different measure of outcome, which is the change in MADRS score, we observed similar results. These findings would suggest that venlafaxine dose might not be a good predictor of change in MADRS scores.

In addition, we observed a weak positive correlation between dose trajectory and change in MADRS score. Few clinical studies have investigated the relationship between the dosing regimen over time (dose trajectory pattern) and change in MADRS score in depressed patients. Since we observed a relationship between clinical outcomes and dose trajectory patterns but not end dose, it is expected that dose trajectory may be more predictive of therapeutic efficacy than end dose, which is commonly reported in literature. To the best of our knowledge, this is the first study that investigate the relationship between venlafaxine dose trajectory and clinical outcomes in depressed patients. This finding is clinically relevant because venlafaxine dose is often titrated based on clinical outcomes as recommended in the product label. Therefore, studies which

investigate the relationship between end dose and clinical outcomes may not accurately assess the impact of the dosing regimen over time on clinical outcomes.

4.4.3 Relationship Between Drug Concentration and Clinical Outcomes

Our study showed that there is a weak positive correlation between drug concentration at week 12 and the change in MADRS scores at the end of study. There are few studies that investigated the association between venlafaxine concentration and the change in MADRS scores in depressed patients. A clinical study by Charlier et al have reported a moderate positive correlation between drug concentration and MADRS scores at week 6 after starting venlafaxine treatment.[167] Although our study evaluated the change in MADRS score, our results were similar to those reported by Charlier et al. before and after adjusting for clinical covariates. In addition, we observed a higher drug concentration at week 1 in patients with a low MADRS trajectory pattern when compared to patients with a high MADRS trajectory pattern. A previous clinical study by Gex-Fabry et al. reported that higher drug concentration at week 2 is associated with early response. Taken together, these studies suggest that achieving a high drug concentration after initiation of treatment may lead to earlier onset of response and/or improved outcomes in depressed patients.

4.4.4 Relationship Between Baseline MADRS and Clinical Outcomes

The relationship between pretreatment depressive symptoms severity (Baseline-MADRS) and clinical outcome has been reported in previous studies.[168, 169] In our study, we observed that

pre-treatment depression severity most consistently predicted our clinical outcomes (response, MADRS trajectory and change in MADRS scores) over a 12-week period. In addition, we observed that increased pre-treatment depression severity was associated with reduced response both before and after adjustment for covariates. Patients with reduced pre-treatment depression score showed an earlier response time and to a greater extent than those with elevated pre-treatment depression score. These findings are consistent with to previous studies that report an association between higher baseline-MADRS score and poor clinical outcomes during antidepressant treatment.[168, 170] Grammer et al have reported that remission were higher in patients with baseline-MADRS of mild to moderate depression when compared to severe depression after the use of repetitive transcranial magnetic stimulation (rTMS) for acute treatment of depression.[170] In addition, Joel et al has shown that patients with baseline MADRS score <27 had greater chance of remission after venlafaxine treatment.[168] Collectively, these findings suggest that pre-treatment depression severity could be utilized by clinicians to help tailor antidepressant treatment. A better understanding of early predictors of clinical response can help reduce the risk for complications of being undertreated, limit the exposure to high doses of drug, and reduce the time to respond.

4.4.5 Early Versus Late Responder

In our study, we report that ~ 25% of subjects responded to target dose of 150 mg/day while others responded to a higher dose of 300 mg/day. These findings are consistent with a previous study which reported that higher doses of venlafaxine are associated with greater response.[171] These findings suggest that higher doses of venlafaxine might be necessary to achieve clinical response in a subset of patients which do not respond to lower doses of venlafaxine.[172]

4.5 Conclusion

In summary, our results showed a positive correlation between drug concentration and change in MADRS scores, but not venlafaxine dose. In addition, our results showed that patients in the low MADRS trajectory had higher drug concentration at an early time point (week 1). Moreover, increased pre-treatment depression severity was associated with reduced response.

Taken together, these studies suggest that venlafaxine dose might not be a good predictor of change in MADRS scores. In addition, achieving a high drug concentration after initiation of treatment may lead to earlier onset of response and/or improved outcomes in depressed patients. In addition, pre-treatment depression severity could be utilized by clinicians to help tailor antidepressant treatment. A better understanding of early predictors of clinical response can help reduce the risk for complications of being undertreated, limit the exposure to high doses of drug and reduce the wait time for response.

5.0 Relationship Between Venlafaxine Dose, Drug Concentration, and Functional Connectivity in the Brain

5.1 Introduction

MDD is a neuropsychiatric disorder which is characterized by emotional and cognitive dysfunction. Recent fMRI studies suggest that MDD is associated with alteration in two important neuronal networks: DMN and ECN[173]. The DMN consist primarily of the following brain regions: posterior cingulate cortex (PCC), medial prefrontal cortex (mPFC), and the inferior parietal lobule (IPL).[174] The DMN is shown to be highly active when subjects are left to think to themselves undisturbed, or during tasks involving self-related processing such as self-referential thoughts. This network is less active when the brain is involved in tasks required cognitive effort.[175,176]

Previous studies have shown that depressed patients demonstrated increased activity of DMN compared to healthy individuals and that activation of DMN in depressed patients was associated with negative bias, increased self-referential thoughts, and rumination.[34] Clinical studies have shown that reduced DMN activity was associated with improved outcomes in patients with depression. For example, a recent study by Wang et al. reported that reduced DMN activity was significantly correlated with symptomatic improvement after 8 weeks of antidepressant treatment.[177] Similarly, Simplicio et al. demonstrated that administration of SSRI (citalopram) in depressed patients reduces negative self-referential processing in mPFC region, which is part of the DMN.[178] Moreover, our lab has demonstrated reduced DMN activity in remitters compared to non-remitters after 12 weeks of venlafaxine treatment in depressed patinets.[35] Taken together,

these studies suggest that improved outcomes in depressed patients on antidepressant therapy may be mediated, at least in part, by a reduction in DMN activity.

The ECN network consists of specific set of regions including anterior cingulate cortex (ACC) and the dorsolateral prefrontal cortex (DLPFC). The ECN is involved in emotion regulation, goal-directed behaviors and complex cognitive task such as working memory and decision making.[35] Previous clinical study have reported reduced activation of ECN in depressed patients when compared to healthy individuals.[36] The reduced activation of ECN in MDD patients has been associated with cognitive dysfunction including, worsen working memory and attention, difficulties in processing information.[35] In addition, clinical studies have suggested that antidepressant treatment may normalize or increase ECN activity in depressed patients and thus improved outcomes. For example, a few studies have demonstrated that SSRI treatment normalized the hypoactivation of the DLPFC region, which is part of the ECN.[179, 180] Similarly, our lab has demonstrated that increased ECN activity in remitters compared to non-remitters after 12 weeks of venlafaxine treatment in depressed elderly patinets.[35] These studies suggest that improved outcomes in depressed patients on antidepressant therapy may be mediated, at least in part, by normalizing or increasing in DMN activity.

Collectively, these studies suggest that antidepressant treatment and improvement in depressive symptoms may be linked to changes within the DMN and ECN. Based on these clinical findings, our lab identified six candidate brain regions within DMN and ECN network that may play a role in the pathophysiology of MDD: the right middle temporal gyrus (rMTG), the left middle temporal gyrus (lMTG), the right inferior frontal gyrus (rIFG) and right supramarginal

gyrus (rSMG) which are brain regions within DMN and right middle temporal gyrus (rMTG) and right precentral gyrus (rPCG) regions within ECN. In Chapter 3, we identified an association between both between venlafaxine dose and drug concentration with clinical outcomes. Therefore, the aim of this chapter is to investigate the association between venlafaxine dose, drug concentration, and functional connectivity in the six candidate brain regions in depressed patients. We expect that venlafaxine dose and/or drug concentration is associated with functional connectivity changes in key brain regions in depressed patients.

5.2 Material and Methods

Detailed description of the study design and participants, dosing regimen, sampling and analytical methods are previously described in detail in chapter 3.

5.2.1 Brain imaging

Functional magnetic resonance imaging (fMRI) scanning was assessed at baseline, placebo, day 1, week 1 and week 12 using methods previously described.[35] Briefly, the scan was performed during resting state; therefore, subjects were instructed to stay awake with their eyes open during the scan. The fMRI scanning was conducted using a 3T Siemens Trio TIM scanner (Munich, Germany) located at the Magnetic Resonance (MR) Research Center at the University of Pittsburgh. Detailed information on fMRI data acquisition and analyses are previously described.[35]

After fMRI scanning and data processing, the correlation between blood-oxygen-level dependent (BOLD) signals of a brain region and a region of interest (ROI) were obtained using Pearson correlation and values, referred to as fMRI scores, were used for statistical analysis. The ROIs for the DMN and ECN were posterior cingulate cortex (PCC) and left dorsolateral prefrontal cortex (dlPFC), respectively. We evaluated four brain DMN functional connectivity including l-MTG-DMN, r-MTG-DMN, r-IFG-DMN, r-SMG-DMN and two ECN functional connectivity including r-MTG-ECN and r-PCG-ECN.

5.2.2 Statistical Analysis

Statistical analyses were performed using IBM SPSS statistical software version 24.0 (SPSS Inc., Chicago, IL, USA). In this study, we investigated the relationship between venlafaxine dose and drug concentration with the change in fMRI score from administration of placebo to week 12. fMRI scores were corrected for baseline measurements and placebo response. Independent variables in the analyses included venlafaxine end dose at week 1 and week 12, dose trajectory overtime, drug concentration at week 1 and week 12. Covariates included in the analysis are patient demographics and baseline clinical characteristics, such as age, gender (M/F), race (C/AA), baseline-MADRS score, depression type (single/recurrent), and baseline mini-mental state examination (MMSE), baseline fMRI score. We treated age, baseline-MADRS, baseline-MMSE, baseline fMRI score as continuous variables. Gender, race and depression type were binary variables. Our goal was to model the relationship between these potential predictors and the change in fMRI at week 12 using regression analysis. For test of association, a comparison between two groups was performed using the student's t-test and Mann-Whitney U-test for normally and non-normally distributed variables, respectively. The association between two continuous variables

was evaluated using Pearson and spearman correlations for normally and non-normally distributed variables, respectively. Two-tailed p-values below 0.05 were regarded as statistically significant in all analyses. For multivariate assessment, linear regression was performed as previously described in chapter 3.

5.3 Results

5.3.1 Description of fMRI Data

The descriptive statistics and test of normality for fMRI scores are shown in Table 39. The histogram distribution of fMRI scores in six candidate brain regions are shown in Figures 25. All fMRI scores were normally distributed.

Table 39: Descriptive statistics and test for normality

fMRI scores	Descriptive statistics				Normality test		
	n	Mean ± SD	Minimum	Maximum	Skewness	Kurtosis	P-value
ΔlMTG-DMN	32	0.074± 0.995	-1.99	2.29	0.414	0.809	0.2
ΔrMTG-DMN	32	0.248±0.773	-1.4	1.47	0.414	0.809	0.2
ΔrIFG-DMN	32	0.209±0.849	-1.73	1.72	0.414	0.809	0.2
ΔrSMG-DMN	32	-0.083±1.24	-3.46	2.68	0.414	0.809	0.2
ΔrMTG-ECN	32	0.234±0.995	-2.13	1.93	0.414	0.809	0.2
ΔrPCG-ECN	32	0.155±0.899	-1.75	2.56	0.414	0.809	0.2

ΔlMTG=change in left middle temporal gyrus; ΔrMTG=change in right middle temporal gyrus; ΔrSMG=change in right supramarginal gyrus; ΔrIFG=change in right inferior frontal gyrus; ΔrPCG= change in right precentral gyrus; DMN= default mode network; ECN= executive control network. Normality test was performed using Kolmogorov-Smirnov test.

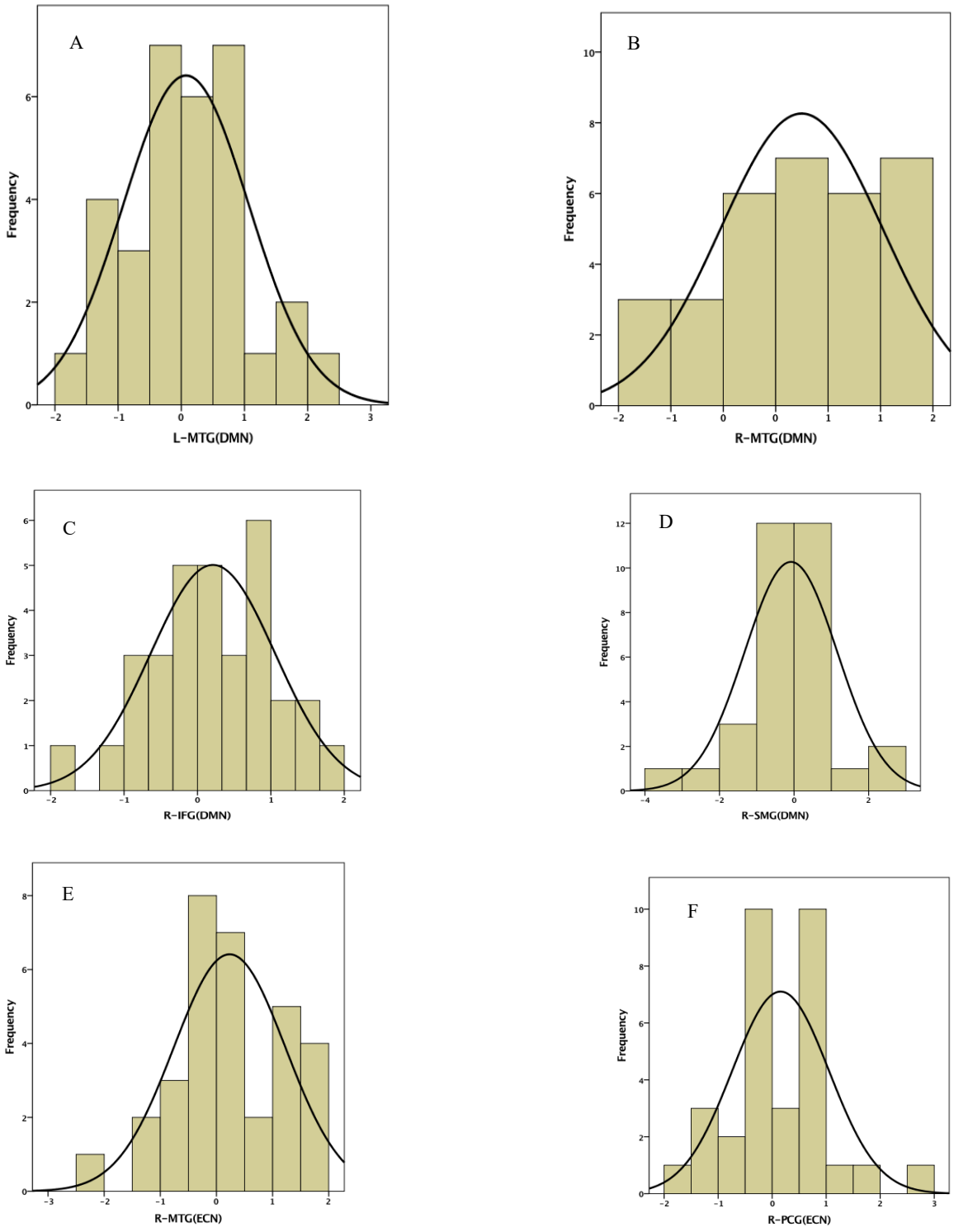


Figure 25: Histogram of six candidate brain regions

Panel A: histogram for the change in left middle temporal gyrus (L-MTG); Panel B: histogram for the change in right middle temporal gyrus (R-MTG); Panel C: histogram for the change in supramarginal gyrus (R-SMG);

Panel D: histogram for the change in right inferior frontal gyrus (R-IFG); Panel E: histogram for the change in right middle temporal gyrus (R-MTG); Panel F: histogram for the change in right precentral gyrus (R-PCG); DMN= default mode network; ECN= executive control network. X-axis represent the change in fMRI scores and Y-axis represent the frequency of samples.

5.3.2 Relationship Between Covariates and Change in fMRI Scores

The relationship between covariates and mean change in fMRI scores in the DMN and ECN at week 12 are shown in Table 40 and Table 41, respectively. At week 12, females had increased Δr-MTG-DMN scores (0.469 ± 0.590) when compared to males (-0.239 ± 0.927, p=0.014). Also, patients with recurrent depression had increased ΔrPCG-ECN scores (0.456 ± 0.827) when compared to patients with single depression (-0.233 ± 0.791, p=0.04). In addition, we observed a weak negative correlation between baseline MADRS scores and ΔrMTG-DMN scores (r= -0.418, p=0.017). Likewise, we observed a weak negative correlation between education and ΔrSMG-DMN scores (r= -0.397, p=0.024).

Table 40: Relationship between covariates and change in fMRI scores

Categorical Covariates									
Covariate	Group	ΔlMTG-DMN		ΔrMTG-DMN		ΔrSMG-DMN		ΔrIFG-DMN	
		Mean ± SD (n)	P-value	Mean ± SD (n)	P-value	Mean ± SD (n)	P-value	Mean ± SD (n)	P-value
Age	≤ 65	0.124±0.942(15)	0.795	0.247±0.754(15)	0.996	-0.005±0.97(15)	0.743	0.414±0.644(15)	0.208
	>65	0.030±1.07(17)		0.248±0.812(17)		-0.153±1.47(17)		.03±0.980(17)	
Gender	Male	-0.374±1.22(10)	0.149	-0.239±0.927(10)	0.014*	0.072±0.881(10)	0.64	0.468±0.809(10)	0.254
	Female	0.278±0.827(22)		0.469±0.590(22)		-0.154±1.39(22)		0.093±0.859(22)	
Race	Caucasian	0.170±1.039(25)	0.31	0.275±0.827(25)	0.705	-0.155±1.26(25)	0.546	0.156±0.874(25)	0.51
	African American	-0.269±0.792(7)		0.147±0.575(7)		0.173±1.23(7)		0.401±0.787(7)	
Depression type	Single	0.217±1.061(10)	0.441	0.148±0.562(10)	0.751	0.450±1.19(10)	0.176	0.62±0.667(10)	0.081
	Recurrent	-0.076±0.905(19)		0.249±0.902(19)		-0.179±1.14(19)		0.033±0.900(19)	
Continous Covariates									
Covariates	n	ΔlMTG-DMN		ΔrMTG-DMN		ΔrSMG-DMN		ΔrIFG-DMN	
		Pearson (r)	p-value	Pearson (r)	p-value	Pearson (r)	P-value	Pearson (r)	P-value
Age (yr)	32	0.004	0.983	-0.024	0.895	0.038	0.838	-0.104	0.571
Education (yr)	32	0.002	0.992	-0.016	0.93	-0.397	0.024*	-0.281	0.119
MMSE baseline	32	0.092	0.615	0.11	0.551	0.219	0.228	0.245	0.176
MADRS baseline	32	-0.244	0.179	-0.418	0.017*	-0.038	0.835	0.092	0.617

Categorical and continuous covariates were compared to fMRI scores in the DMN (ΔlMTG-DMN, ΔrMTG-DMN ΔrSMG-DMN, and ΔrIFG-DMN) using Student's t-test and Pearson correlation, respectively. Statistical significance established at p<0.05. MMSE= Mini Mental State Examination, MADRS= Montgomery and Asberg Depression Rating Scale, yr=year. ΔlMTG=change in left middle temporal gyrus; ΔrMTG=change in right middle temporal gyrus; ΔrIFG=change in right inferior frontal gyrus; ΔrSMG=change in right supramarginal gyrus; DMN= default mode network.

Table 41: Relationship between covariates change in fMRI scores

Categorical Covariates					
Covariate	**Group**	**ΔrMTG-ECN**		**ΔrPCG-ECN**	
		Mean ± SD (n)	**P-value**	**Mean ± SD (n)**	**P-value**
Age	≤ 65	0.168±1.08(15)	0.732	0.027±0.77(15)	0.458
	>65	0.291±0.945(17)		0.268±1.008(17)	
Gender	Male	0.287±1.156(10)	0.842	-0.015±0.725(10)	0.478
	Female	0.209±0.942(22)		0.233±0.973(22)	
Race	Caucasian	0.212±1.047(25)	0.821	0.278±0.868(25)	0.146
	African American	0.311±0.85(7)		-0.283±0.932(7)	
Depression type	Single	0.27±1.206(10)	0.941	-0.233±0.791(10)	0.04*
	Recurrent	0.24±0.932(19)		0.456±0.827(19)	
Continous Covariates					
Covariates	**n**	**ΔrMTG-ECN**		**ΔrPCG-ECN**	
		Pearson (r)	**P-value**	**Pearson (r)**	**P-value**
Age (yr)	32	0.179	0.327	-0.077	0.675
Education (yr)	32	0.139	0.448	0.176	0.335
MMSE baseline	32	0.261	0.149	0.022	0.905
MADRS baseline	32	-0.05	0.786	0.233	0.199

Categorical and continuous covariates were compared to fMRI scores in the ECN (Δr-MTG-ECN and ΔrPCG-ECN) using Student's t-test and Spearman correlation, respectively. Statistical significance was established at $p<0.05$. MMSE= Mini Mental State Examination, MADRS= Montgomery and Asberg Depression Rating Scale, yr=year. ΔrMTG=change in right middle temporal gyrus; ΔrPCG= change in right precentral gyrus; ECN= executive control network.

5.3.3 Relationship Between Venlafaxine Dose and Change in fMRI Scores

5.3.3.1 Relationship Between Change in fMRI Scores and Venlafaxine Dose at Week 12

The correlation between venlafaxine dose and change in fMRI scores at week 12 is shown in Table 42. We observed a weak negative correlation between ΔrMTG-DMN signal and venlafaxine end dose at week 12 (r= -0.470, p=0.007). However, this relationship failed to remain significant after controlling for covariates.

Table 42: Relationship between venlafaxine end dose and change in fMRI scores at week 12

fMRI scores	End dose at week 12			
	Unadjusted		Adjusted	
	Spearman correlation (n)	p-value	Standardized beta coefficient (95%CI)	p-value
ΔlMTG-DMN	-0.245 (32)	0.177	-0.045 (-0.007 to 0.006)	0.828
ΔrMTG-DMN	-0.470 (32)	0.007*	-0.251 (-0.008 to 0.002)	0.189
ΔrIFG-DMN	0.222 (32)	0.222	0.029(-0.005 to 0.006)	0.889
ΔrSMG-DMN	-0.042 (32)	0.817	-0.220 (-0.010 to 0.003)	0.225
ΔrMTG-ECN	0.096 (32)	0.602	0.009 (-0.007 to 0.006)	0.965
ΔrPCG-ECN	-0.33 (32)	0.069	-0.0002 (-0.005 to 0.005)	0.999

The association between venlafaxine end dose at week 12 and change in fMRI scores at week 12 was assessed using Spearman correlation. Adjusted p-value was obtained after performing regression analysis controlling for covariates. Statistical difference was established at *p<0.05.

5.3.3.2 Change in fMRI Scores at Week 12 by Dose Trajectory Groups

The mean change in fMRI scores at week 12 in the dose trajectory groups is shown in Table 43. Patients in the high dose trajectory group had lower ΔrMTG-DMN scores (-0.060 ± 0.701) when compared to patients in the low dose trajectory (0.555 ± 0.735, p=0.022). However, this relationship failed to remain significant after controlling for covariates.

Table 43: Change in fMRI scores at week 12 by dose trajectory groups

fMRI scores	Low dose trajecotry	High dose trajectory	Unadjusted P-value	Adjusted	
	Mean ± SD (n)	Mean ± SD (n)		Standardized beta coefficient (95%CI)	p-value
ΔlMTG-DMN	0.374 ± 1.004 (16)	-0.225 ± 0.922 (16)	0.089	-0.117 (-0.977 to 0.540)	0.557
ΔrMTG-DMN	0.555 ± 0.735 (16)	-0.060 ± 0.701 (16)	0.022*	-0.279 (-1.030 to 0.162)	0.146
ΔrIFG-DMN	0.177 ± 0.968 (16)	0.242 ± 0.743 (16)	0.833	-0.057(-0.852 to 0.657)	0.791
ΔrSMG-DMN	-0.144 ± 1.092 (16)	-0.023 ± 1.411 (16)	0.787	0.064 (-0.881 to 1.179)	0.767
ΔrMTG-ECN	0.081 ± 0.964 (16)	0.386 ± 1.034 (16)	0.394	-0.067 (-0.950 to 0.685)	0.74
ΔrPCG-ECN	0.181 ± 0.925 (16)	0.129 ± 0.901 (16)	0.875	-0.150 (-0.888 to 0.378)	0.412

The change in fMRI scores at week 12 was compared to low and high dose trajectory groups using Student's t-test. Adjusted p-value was obtained after performing regression analysis controlling for covariates. Statistical difference was established at *p<0.05.

5.3.4 Relationship Between Drug Concentration and Change in fMRI Scores

The relationship between change in fMRI scores and drug concentration at week 1 and week 12 is shown in Table 44 and Table 45, respectively. We did not observe an association between change in fMRI scores at week 12 and drug concentration at week 1 and week 12.

Table 44: Relationship between drug concentration at week 1 and change in fMRI scores

fMRI scores	Total concentration at week 1 (ng/ml)			
	Unadjusted		Adjusted	
	Pearson correlation (n)	p-value	Standardized beta coefficient (95%CI)	p-value
ΔlMTG-DMN	0.109 (29)	0.574	-0.047 (-0.005 to 0.004)	0.824
ΔrMTG-DMN	0.231 (29)	0.228	0.087 (-0.002 to 0.004)	0.658
ΔrIFG-DMN	-0.046 (29)	0.814	0.142 (-0.002 to 0.005)	0.48
ΔrSMG-DMN	-0.062(29)	0.749	-0.119 (-0.006 to 0.003)	0.547
ΔrMTG-ECN	-0.039(26)	0.841	0.26 (-0.001 to 0.007)	0.188
ΔrPCG-ECN	-0.119(29)	0.537	0.012 (-0.003 to 0.004)	0.948

The association between drug concentration at week 1 and change in fMRI scores at week 12 was assessed using Pearson correlation. Adjusted p-value was obtained after performing regression analysis controlling for covariates. Statistical difference was established at *p<0.05.

Table 45: Relationship between drug concentration at week 12 and change in fMRI scores

fMRI scores	Total concentration at week 12 (ng/ml)			
	Unadjusted		Adjusted	
	Pearson correlation (n)	p-value	Standardized beta coefficient (95%CI)	p-value
ΔlMTG-DMN	-0.036 (31)	0.849	0.047 (-0.001 to 0.002)	0.818
ΔrMTG-DMN	-0.189 (31)	0.308	-0.054 (-0.001 to 0.001)	0.777
ΔrIFG-DMN	0.299 (31)	0.103	0.180 (- 0.001 to 0.002)	0.312
ΔrSMG-DMN	-0.019(31)	0.921	-0.179 (-0.003 to 0.001)	0.327
ΔrMTG-ECN	-0.193 (31)	0.299	-0.113 (-0.002 to 0.001)	0.557
ΔrPCG-ECN	-0.189 (31)	0.309	0.047 (-0.001 to 0.001)	0.787

The association between drug concentration at week 12 and change in fMRI scores at week 12 was assessed using Pearson correlation. Adjusted p-value was obtained after performing regression analysis controlling for covariates. Statistical difference was established at *p<0.05.

5.3.5 Relationship Between Change in fMRI Scores and Clinical Outcomes

5.3.5.1 Change in fMRI Scores by MADRS Trajectory Group

The mean change in fMRI scores in the MADRS trajectory groups is shown in Table 46. Patients in the low MADRS trajectory group had reduced ΔrIFG-DMN scores (0.026 ± 1.094) when compared to patient in the high MADRS trajectory group (1.069 ± 0.515, p=0.042). In addition, patients in the low MADRS trajectory group had increased ΔrMTG-DMN scores (0.619 ± 0.686) when compared to patient in the high MADRS trajectory group (-0.566 ± 0.761, p=0.014).

Table 46: Mean fMRI scores by MADRS trajectory groups

fMRI scores	MADRS Trajectory			
	Low	Moderate	High	P-value
	Mean ± SD (n)	Mean ± SD (n)	Mean ± SD (n)	
ΔlMTG-DMN	0.149 ± 1.241(10)	0.192 ±0.855(17)	-0.476 ± 0.915(5)	0.415
ΔrMTG-DMN	0.619±0.686(10)^	0.268±0.673(17)	-0.566±0.761(5)	0.014*
ΔrIFG-DMN	0.026± 1.094(10)^	0.065±0.619(17)	1.069±0.515 (5)	0.042*
ΔrSMG-DMN	-0.138±1.459(10)	-0.228±1.237(17)	0.516±0.719(5)	0.508
ΔrMTG-ECN	0.108±0.806(10)	0.141±1.126(17)	0.799±0.821(5)	0.395
ΔrPCG-ECN	0.284±1.011(10)	0.247±0.847(17)	-0.414±0.779(5)	0.312

The mean change in fMRI scores at week 12 was compared to low, moderate and high MADRS trajectory groups using ANOVA test. Bonferroni's post hoc analysis was performed comparing the high MADRS trajectory (reference group) with low MADRS trajectory and moderate MADRS trajectory. Statistical difference was established at ^p<0.05. ΔlMTG=change in left middle temporal gyrus; ΔrMTG=change in right middle temporal gyrus; ΔrIFG=change in right inferior frontal gyrus; ΔrSMG=change in right supramarginal gyrus; ΔrPCG= change in right precentral gyrus. DMN= default mode network; ECN= executive control network.

5.3.5.2 Change in fMRI Scores by Clinical Response

The mean change in fMRI scores in clinical response groups is shown in Table 47. The responder group had lower ΔrFIG-DMN scores (-0.022 ± 0.914) when compared to the non-responder group (0.549 ± 0.632, p=0.018). In addition, the responder group had higher ΔrMTG-DMN scores (0.0508 ± 0.723) when compared to the non-responder group (-0.133 ± 0.702, p=0.038).

Table 47: Relationship between change in fMRI scores and clinical response

fMRI scores	Non-Responders	Responders	Unadjusted p-value	Adjusted p-value	OR (95% CI)
	Mean ± SD (n)	Mean ± SD (n)			
ΔlMTG-DMN	-0.221 ± 0.694 (13)	0.276 ± 1.131 (19)	0.169	0.289	0.441 (0.097 to 2.001)
ΔrMTG-DMN	-0.133 ± 0.702 (13)	0.508 ± 0.723 (19)	0.019*	0.038*	15.022 (1.167 to 193.392)
ΔrIFG-DMN	0.549 ± 0.632 (13)	-0.022 ± 0.914 (19)	0.06	0.018*	0.003 (0.00003 to 0.383)
ΔrSMG-DMN	0.194 ± 1.083 (13)	-0.273 ± 1.336 (19)	0.304	0.454	0.301 (0.013 to 6.956)
ΔrMTG-ECN	0.629 ± 0.990 (13)	-0.037 ± 0.929 (19)	0.062	0.131	0.018 (0.0001 to 3.296)
ΔrPCG-ECN	-0.243 ± 0.894 (13)	0.428 ± 0.815 (19)	0.035*	0.175	2.783 (0.634 to 12.207)

The mean change in fMRI scores at week 12 was compared to responder and non-responder (reference group) using Student's t-test. Regression analysis was performed after adjusting for covariates. Statistical difference was established at *p<0.05. Odd ratio (OR); 95% confidence interval (95%CI). ΔlMTG=change in left middle temporal gyrus; ΔrMTG=change in right middle temporal gyrus; ΔrIFG=change in right inferior frontal gyrus; ΔrSMG=change in right supramarginal gyrus; ΔrPCG= change in right precentral gyrus. DMN= default mode network; ECN= executive control network.

5.3.5.3 Relationship Between Change in fMRI Scores and Change in MADRS Scores

The correlation between change in fMRI scores and change in MADRS scores is shown in Table 48. The correlation between ΔrFIG-DMN scores and change in MADRS scores are shown in Figure 26. After controlling for covariates, we observed a weak positive correlation between ΔrFIG-DMN scores and change in MADRS scores (r=0.346, p=0.001) and a very weak positive correlation between ΔrMTG-ECN scores and change in MADRS scores (r=0.138, p=0.049).

Table 48: Relationship between change in fMRI scores and change in MADRS scores

fMRI scores	Change in MADRS scores			
	Unadjusted		Adjusted	
	Pearson correlation (n)	p-value	Standardized beta coefficient (95%CI)	p-value
ΔlMTG-DMN	-0.131 (32)	0.476	-0.088 (-4.123 to 2.535)	0.626
ΔrMTG-DMN	-0.116 (32)	0.527	-0.263 (-6.349 to 0.678)	0.109
ΔrIFG-DMN	0.346 (32)	0.053	0.540 (2.540 to 8.168)	0.001*
ΔrSMG-DMN	-0.062 (32)	0.736	0.027 (-2.553 to 2.943)	0.884
ΔrMTG-ECN	0.138 (32)	0.451	0.299 (0.007 to 5.035)	0.049*
ΔrPCG-ECN	-0.128 (32)	0.484	-0.113 (-4.754 to 2.528)	0.533

The association between change in fMRI scores at week 12 and change in MADRS scores at week 12 was assessed using Pearson correlation. Adjusted p-value was obtained after performing regression analysis controlling for covariates. Statistical difference was established at *p<0.05. ΔlMTG=change in left middle temporal gyrus; ΔrMTG=change in right middle temporal gyrus; ΔrIFG=change in right inferior frontal gyrus; ΔrSMG=change in right supramarginal gyrus; ΔrPCG= change in right precentral gyrus. DMN= default mode network; ECN= executive control network.

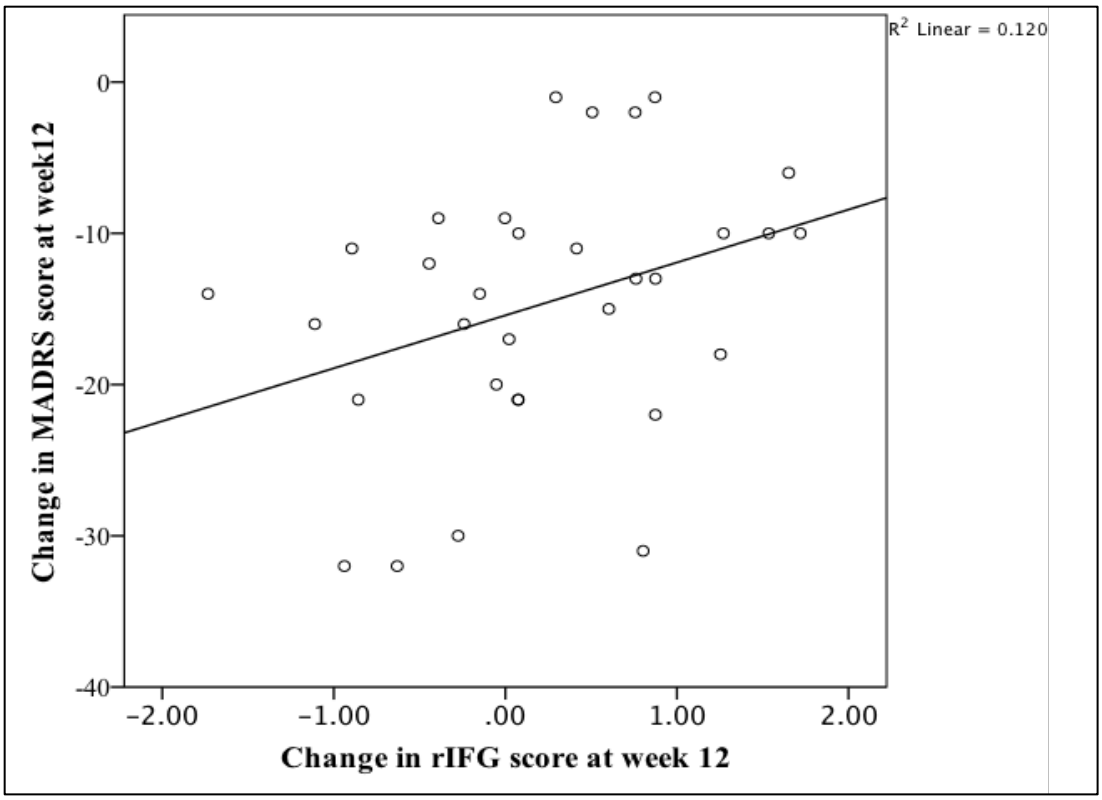


Figure 26: Correlation between change in rIFG-DMN and change in MADRS scores at week 12

The scatter plot represents the mean individual values; continuous line is the regression line.

5.4 Discussion

This clinical study is the first to investigate the relationship between venlafaxine dose, drug concentration and functional connectivity in patients with MDD. Our results demonstrate that changes in DMN functional connectivity is associated with improved clinical outcomes. Also, there was no association between functional connectivity in our candidate brain regions and neither venlafaxine dose and drug concentration in patients with MDD.

5.4.1 Relationship Between Functional Connectivity in the Brain and Clinical Outcomes

Previous clinical studies reported an association between DMN functional connectivity and improvement of depressive symptoms following antidepressant treatment. However, there are few clinical studies investigating these relationships in patients on venlafaxine treatment. To the best of our knowledge, we are the first to investigate the relationship between venlafaxine dose, drug concentration and functional connectivity in the brain in depressed patients.

In our study, we observed an association between reduced ΔrIFG-DMN connectivity and improved clinical outcomes. Specifically, decreased ΔrIFG-DMN connectivity was observed in patients that achieved clinical response and in patients in the low MADRS trajectory group. Also, there was a weak positive correlation between ΔrIFG-DMN connectivity and lower change in MADRS (worse outcomes). Although our study evaluated the change in connectivity from placebo to week 12, our results are consistent with previously published data demonstrating an association between decreased rIFG-DMN connectivity and improved outcomes, as measured by clinical remission, in patients with MDD.[35]

Also, previous studies in depressed patients on antidepressant therapy suggest that improved outcomes may be mediated, at least in part, by a reduction in DMN connectivity.[177] Wang et al reported a reduction in the bilateral dorsomedial prefrontal cortex (dmPFC) connectivity, a subregion of the DMN, after 8-week treatment of SSRI-escitalopram. This study reported a positive correlation between dmPFC connectivity changes and symptoms improvement as measured using Hamilton depression rating scale (HAMD).[177] Conversely, some clinical studies did not observe an association between changes in DMN connectivity and symptoms improvement during antidepressant treatment.[181, 182] Posner et al. investigated the effect of antidepressant SNRI-duloxtrine on DMN connectivity in patients with persistent depression.[182] Although the study reported reduced DMN connectivity after 10-week treatment with duloxtrine, there was no correlation between DMN connectivity changes and symptoms improvement as measured using HAMD scale.[182] A possible explanation for the lack of correlation between connectivity changes and symptoms improvement could be that normalization of DMN may lead to improvement in a specific symptom domain (ie. rumination) rather than the full range of depressive symptoms indexed by the HAMD. Recent studies have shown that DMN activity in patients with MDD correlates with behavioral measures of rumination.[34, 183] Collectively, these data suggest that reduced of DMN connectivity may impact clinical outcomes in MDD patients on antidepressant therapy.

On the other hand, we observed association between increased ΔrMTG-DMN connectivity and improved outcomes. Specifically, increased ΔrMTG-DMN connectivity was observed in patients that achieved clinical response and in patients in the low MADRS trajectory group. These results are consistent with previously published data demonstrating an association between

increased ΔrMTG-DMN connectivity and improved outcomes, as measured by clinical remission, in patients with MDD.[35] However, we did not observed a correlation between ΔrMTG-DMN connectivity and change in MADRS. The lack of correlation might suggest that functional connectivity between ΔrMTG-DMN is a neuronal correlate of the magnitude of response to antidepressants and not necessary of MDD severity. However, clinical studies have reported decreased DMN connectivity was associated with improved outcomes in depressed patients.[182] Possible explanation for the observed increased in DMN connectivity could be that changes in functional connectivity after antidepressants may occur in different regions than those which predict the treatment response. Furthermore, since there are multiple regions which constitute the DMN including medial prefrontal cortex (mPFC) and the inferior parietal lobule (IPL), it is possible that neuronal connectivity in one of these regions may play a dominant role in improving clinical outcomes with antidepressant therapy. Also, there may be complex interactions such as rebalancing of the DMN with antidepressant therapy in patients with MDD as previously described.[35] As such, the relationship between DMN connectivity and clinical outcomes in depressed patients requires further study.

5.4.2 Relationship Between Change in fMRI and Dose/Drug Concentration

To the best of our knowledge, this is the first study to investigate the relationship between venlafaxine dose, drug concentration, and changes in functional connectivity in the brain in depressed patients. Our lab has previously demonstrated that venlafaxine treatment induced alteration in functional connectivity of DMN and ECN, but we did not assess the relationship between venlafaxine dose and/or concentration on functional connectivity. In this study, we did

not observe an association between functional connectivity in our candidate brain regions and neither venlafaxine dose and drug concentration in depressed patients.

There are several possible explanations these observed relationships. First, it is possible that the effect of venlafaxine dose and drug concentration on clinical outcomes are not mediated through functional connectivity. In this case, drug concentration and functional connectivity in the brain may independently impact treatment response through different pathways. Conversely, it is possible the relationship between functional connectivity in the brain and clinical outcomes may be mediated by drug concentration at the site of action in the CNS, but our study measured drug concentration in the plasma. However, it is important to note that previous studies have reported a strong correlation between venlafaxine drug concentration in plasma and cerebrospinal fluid (CSF) in depressed patients.[184] Drug concentration in CSF might not represent the concentration at the site of action, but it may reflects availability of drug in the brain. Another potential explanation could be related to the timing of fMRI measures as earlier or later time points might correlate better with drug concentration. Similarly, our study evaluated drug concentration at single time points (week 1 and week 12), whereas full concentration-time profile may be more informative for these analyses. In this case, modeling and simulation tools, such as population PK, could be utilized to predict concentration values over time for additional analysis. Thus, additional studies are needed to further investigate the relationships between venlafaxine dose, drug concentration, and functional connectivity in the brain in depressed patients.

In addition, there are very few clinical studies investigating the targets and/or mechanisms for antidepressant treatment and functional connectivity. Venlafaxine is both a serotonin and

norepinephrine reuptake inhibitor (SNRIs) and selectivity of the inhibition is concentration-dependent. Due to the 30-fold higher affinity for the reuptake inhibition of serotonin compared to norepinephrine, venlafaxine inhibition of serotonin reuptake precedes norepinephrine reuptake.[185,186] Moreover, a clinical study by Debonnel et al. reported that venlafaxine acted as a selective serotonin reuptake inhibitor at low doses (75 mg/day) and a dual serotonin reuptake and norepinephrine reuptake inhibitor at higher doses (225 and 375 mg/day).[187] Although inhibition of either transporter system could be responsible for the normalizing effect of venlafaxine on functional connectivity, recent studies have suggested that neuronal activity in the DMN is affected by the serotonin system.[188] Future studies are needed to directly determine if inhibition of serotonin reuptake, norepinephrine reuptake, or both processes are responsible for normalization of DMN connectivity after antidepressant treatment.

5.5 Conclusion

In our study, we observed an association between improved clinical outcomes and both decreased and increased ΔrIFG-DMN and ΔrMTG-DMN functional connectivity, respectively. In addition, we did not observe an association between functional connectivity in our candidate brain regions and neither venlafaxine dose and drug concentration in depressed patients. Taken together, these data suggest that alteration of DMN connectivity may impact clinical outcomes in MDD patients on antidepressant therapy. Also, it is possible that the effect of venlafaxine dose and drug concentration on clinical outcomes are not mediated through functional connectivity. Thus, additional studies are needed to further investigate the relationships between venlafaxine dose, drug concentration, and functional connectivity in the brain in depressed patients.

6.0 Relationship Between Venlafaxine Dose, Drug Concentration, Brain Functional Connectivity, and Clinical Outcomes using Path Analysis

6.1 Introduction

As previously described in chapter 1, path analysis offers several advantages over regression method. Path analysis helps to quantify the direct and indirect effects on a dependent variable simultaneously. Since we are interested in analyzing a longitudinal study with multiple measures of clinical outcomes, we utilized path analysis to investigate the effect of venlafaxine dose, drug concentration, and functional connectivity in the brain on clinical outcomes simultaneously. To the best of our knowledge, we are the first to investigate these relationships in depressed patients using path analysis.

We developed a theoretical model that describe the relationship between venlafaxine dose, drug concentration, functional connectivity and clinical outcomes (Figure 27). In this model, we hypothesized that effect of venlafaxine dose on functional connectivity in the brain and clinical outcome is mediated through drug concentration. As discussed in chapter 4, the conventional treatment of MDD requires dose titration based on clinical response and tolerability.[156] Despite dose titration, MDD patients on venlafaxine treatment have demonstrated variable response rates and tolerability issues.[10, 157, 158] The underlying factors contributing to the variability in clinical outcomes and tolerability issues in MDD patients on venlafaxine treatment are not well understood.[159] However, some studies report that patient demographics and baseline clinical characteristics, such as age, sex, race, and BMI, weight, and lifestyle factors have been

reported to influence the PK and/or PD of venlafaxine in depressed patients, which may alter individual drug concentration.[152] Therefore, variability in the treatment response to venlafaxine could be related to inter-individual variability in drug concentration. Although venlafaxine drug concentration in plasma is not a direct measurement of drug concentration at the site of action, published studies report a significant correlation between venlafaxine drug concentration in plasma and CSF.[189] Since a drug's effect is related to drug concentration at the site of action, it is expected that not only venlafaxine dose, but also venlafaxine drug concentration in plasma, will be associated with functional connectivity in the brain and clinical outcomes. Based on these findings, we developed a theoretical model to describe the relationship between venlafaxine dose, drug concentration and clinical outcomes at each time point simultaneously.

Hypothesis

a) Venlafaxine dose is directly associated with drug concentration.
b) Venlafaxine dose is directly associated with functional connectivity in the brain and clinical outcomes.
c) The effect of venlafaxine dose on functional connectivity in the brain and clinical outcomes is mediated through drug concentration.

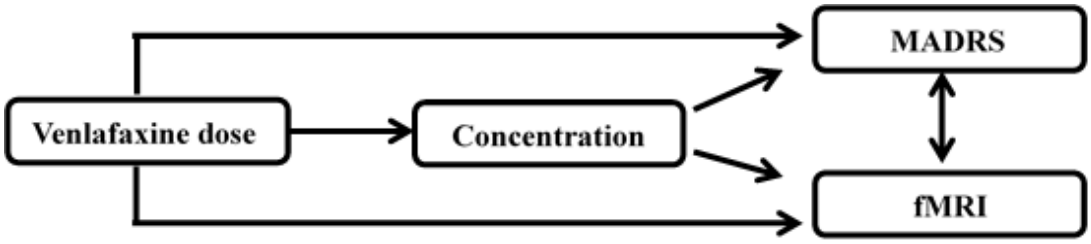


Figure 27:Theoretical model describe the relationship between venlafaxine dose, drug concentration, functional connectivity (fMRI) in the brain and clinical outcome (MADRS).

6.2 Material and Methods

Path analysis was performed using analysis of moment structures (AMOS version 24.0). Based on our predefined research questions, we built a theoretical model that describe the direct and indirect relationship between our variables which includes venlafaxine dose, drug concentration and clinical outcomes at each time point. Then we tested this model using path analysis approach. We obtained estimate and significant levels for each parameter. The goodness-of-fit were assessed using chi-square statistics, which provides a test if the null hypothesis that the theoretical model fits the data. The criteria for good model fit were chi-square ($\chi 2$) >0.05. Other model diagnostic criteria include the following: Goodness-of-fit index (GFI) statistic ≥ 0.95 and root mean square error of approximation (RMSEA) of ≤ 0.05. We simplified our model by eliminating paths not significant in the model and only paths supported by the data are remained in the final model. Finally, we reported the estimates and significant levels of correlation and regression parameters from the fit model. Direct and indirect effects of venlafaxine dose, drug concentration on clinical outcomes were calculated using the standardized regression weights of each pathway.

To specify the paths among the variables in the model, we used single-headed arrows to represent direct relationships and dual-headed arrows to represent bidirectional relationships (correlations). The paths between functional connectivity in the brain and MADRS is bidirectional, all the other paths among the variable are direct. The significance of the path is represented by the standardized regression beta coefficient associated with each of the path. In addition, each path has an R^2 associated with it, which represent the percentage of variance explained by the variables.

6.3 Results

6.3.1 Correlation Coefficients

Table 49 shows the correlation coefficient matrix of the observed variables. MADRS score at week 1 is positively correlated with baseline MADRS score ($r=0.684$, $p<0.01$). MADRS score at week 12 is positively correlated with MADRS score at week 12 ($r=0.466$, $p<0.01$). Baseline rIFG-DMN is negatively correlated with baseline-MADRS score ($r=-0.522$, $p<0.01$) and MADRS score at week 1 ($r=-0.454$, $p<0.01$). End dose at week 12 is positively correlated with MADRS score at week 1 ($r=0.361$, $p<0.05$) and MADRS score at week 12 ($r=0.424$, $p<0.01$). Drug concentration at week 1 is negatively correlated with MADRS score at week 12 ($r=0.361$, $p<0.05$) and positively correlated with dose at week 1 ($r=0.317$, $p<0.05$). Drug concentration at week 12 is positively correlated with MADRS score at week 12 ($r=0.402$, $p<0.01$) and dose at week 12 ($r=0.751$, $p<0.01$).

Table 49: Correlation coefficient matrix of the measured variables

Variables	1	2	3	4	5	6	7	8	9	10
1.Baseline MADRS score	1									
2.MADRS score at week 1	0.684**	1								
3.MADRS score at week 12	0.271	0.466**	1							
4.Baseline rIFG-DMN score	-0.522**	-0.454**	-0.129	1						
5.rIFG-DMN score at week 1	0.135	0.022	0.303	-0.161	1					
6.rIFG-DMN score at week 12	0.124	0.091	0.242	0.066	-0.255	1				
7.End dose at week 1	0.016	-0.04	-0.156	-0.055	-0.131	-0.136	1			
8.End dose at week 12	0.278	0.361*	0.424**	-0.063	0.191	0.146	0.175	1		
9.Total concentration at week 1	-0.244	-0.233	-0.314*	-0.051	-0.189	0.51	0.317*	-0.222	1	
10.Total concentration at week 12	0.085	0.126	0.402**	0.092	0.133	0.23	-0.002	0.751**	0.214	1

** Correlation is significant at the 0.01 level

*Correlation is significant at the 0.05 level

6.3.2 Final Model

The final model describing the relationship between baseline clinical factors, venlafaxine dose, drug concentration, functional connectivity in the brain and depression severity at the end of study is shown in Figure 28 and Table 50. The path model had a good fit with the data (χ^2=34.3, df=33, p= 0.404, GFI=0.992, RMSEA=0.028).

6.3.2.1 Model Testing

(a) Direct Effect

In this model, baseline-MADRS score (β= 0.700, p<0.001) had positive direct effect on MADRS score at week 1. Baseline-MADRS score accounted for 49% of the variance of MADRS score at week 1. MADRS score at week 1 (β= 0.339, p<0.001), drug concentration at week 12 (β=

0.410, p<0.001), and rIFG-DMN score at week 1 (β= 0.335, p<0.001), had positive direct effect on MADRS score at week 12. Drug concentration at week 1(β= -0.412, p<0.001) had negative direct effect on MADRS score at week 12. These explanatory variables accounted for 40% of the variance of MADRS score at week12. Drug concentration at week 12 (β= 0.403, p=0.001) had positive direct effect on rIFG-DMN score at week 12. Drug concentration at week 12 accounted for 16% of the variance of rIFG-DMN score.

End dose at week 1 (β= 0.318, p=0.015) had direct positive effect on drug concentration at week 1 and this explanatory variable account for 10% of the variance of drug concentration at week 1. End dose at week 12 (β= 0.763) and drug concentration at week 1 (β= 0.427, p<0.001) had direct positive effect on drug concentration at week 12. These explanatory variables account for 76% of the variance of drug concentration at week 12. At baseline and week 12, both MADRS score and rIFG-DMN score were intercorrelated.

(b) Indirect Effect

End dose at week 1 is indirectly related to MADRS score at week 12 (mediated by drug drug concentration at week1, β= -0.075, p=0.013). Also, end dose at week 1 is indirectly related to rIFG-DMN score at week 12 (mediated by drug concentration at week12, β= 0.055, p=0.006). End dose at week 1 is indirectly related to drug concentration at week 12 (mediated by drug concentration at week1, β= 0.136, p=0.010). Baseline-MADRS score is indirectly related to MADRS score at week 12 (mediated by MADRS score at week 1, β= 0.237, p=0.006). Similarly, end dose at week 12 is indirectly related to MADRS score at week 12 (mediated by drug concentration at week12, β= 0.313, p=0.009). End dose at week 12 is indirectly related to rIFG-

DMN score at week 12 (mediated by drug concentration at week12, β= 0.307, p=0.004). Drug concentration at week 1 is indirectly related to MADRS score at week 12 (mediated by drug concentration at week 12, β= 0.175, p=0.005). Drug concentration at week 1 is indirectly related to rIFG-DMN score at week 12 (mediated by drug concentration at week 12, β= 0.172, p=0.003).

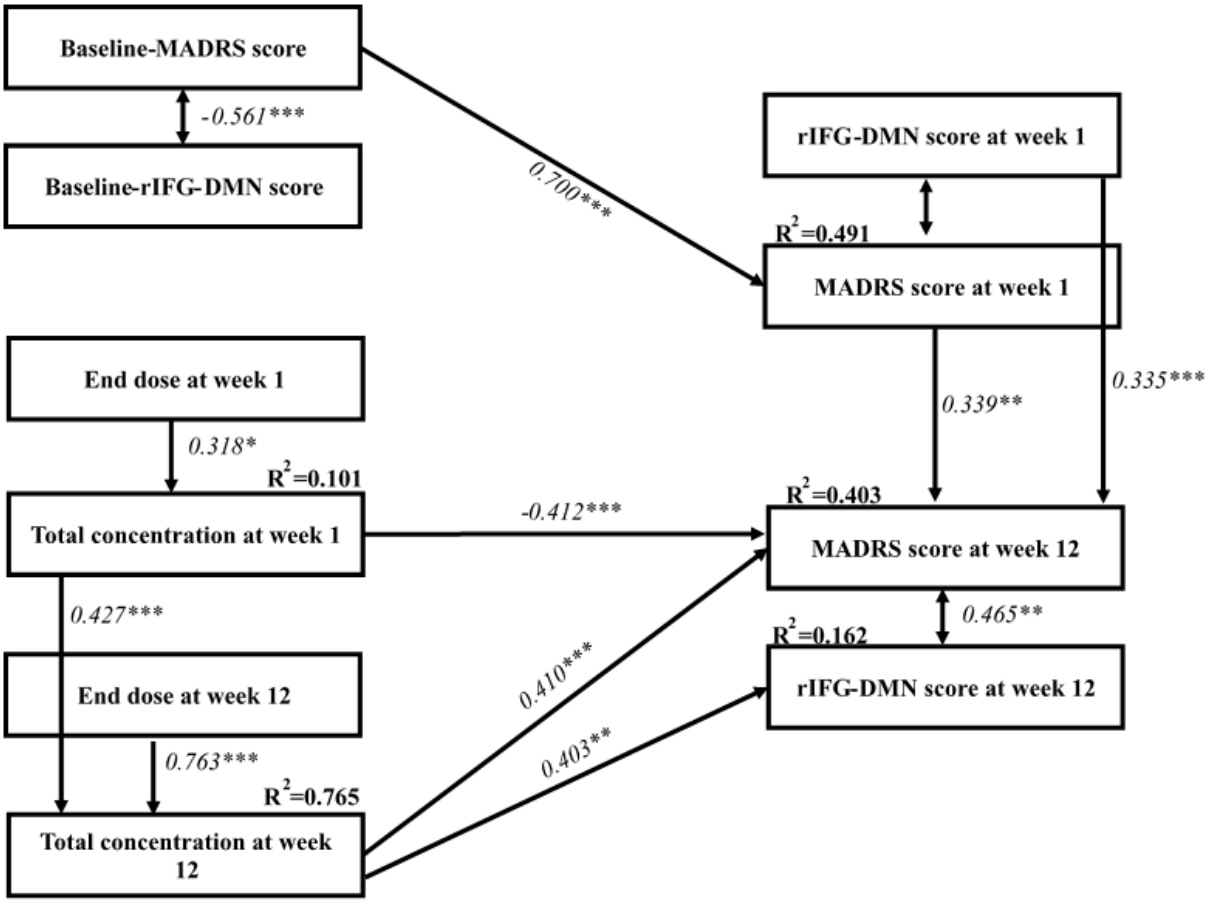


Figure 28: Final Path Model

A path analysis model of the relationship among baseline clinical factors, venlafaxine dose, drug concentration, depressive symptoms severity at week 1, and change in brain functional connectivity at week 1, and final change in depressive symptoms severity. The number shown next to single-headed and double-headed arrows correspond to standardized regression weights. The number in bold above dependent variable represent the square multiple correlation (R^2). *p<0.05, **p<0.01, ***p<0.001

Table 50: Estimated standardized path coefficient, p-value and 95% CI based on the final model

Independent variable	Path	Dependent variable	Standardized regression coefficient	P-value	95% CI
Dose at week1	→	Concentration at week1	0.318	0.02	0.032 to 0.593
Dose at week12	→	Concentration at week12	0.763	0.013	0.674 to 0.842
Concentration at week1	→	Concentration at week12	0.427	0.009	0.265 to 0.548
Concentration at week12	→	MADRS at week12	0.41	0.011	0.157 to 0.651
Concentration at week12	→	rIFG-DMN at week12	0.403	0.004	0.147 to 0.685
rIFG-DMN at week1	→	MADRS-week 12	0.335	0.004	0.118 to 0.562
Concentration at week1	→	MADRS-week 12	-0.412	0.007	-0.602 to -0.229
Baseline-MADRS	→	MADRS-week 1	0.7	0.009	0.530 to 0.801
MADRS at week1	→	MADRS-week 12	0.339	0.012	0.091 to 0.535

6.4 Discussion

This clinical study is the first to investigate the relationship between venlafaxine dose, drug concentration, changes in neuronal connectivity, and clinical outcomes in depressed patients using path analysis. As expected, our study found that pre-treatment depression severity had a direct effect on depression severity at week 1, which in turn showed a direct effect on depression severity at week 12. Also, early changes in functional connectivity of rIFG-DMN at week 1 predicts depression severity at week 12. In addition, path analysis revealed that dose at week 1 and dose at week 12 both exhibit an indirect effect on depression severity at week 12, which is mediated through drug concentration at week 1 and week 12, respectively. Furthermore, there was a moderate correlation between rIFG-DMN and depression severity both before treatment and at week 12.

Previously published studies have investigated the relationship between depression severity scores and/or clinical outcomes at various time points during treatment. A clinical study by Muzina et al showed that MADRS scores at week 2 was a significant predictor of remission after 8-week of antidepressant treatment.[190] Although our study evaluated MADRS at week 12, our results were similar to those reported by Muzina et al. In fact, we reported a similar relationship could be observed, but at earlier time point. As we expected, the association between baseline MADRS and MADRS at week 1 were stronger compared to association between MADRS week 1 and MADRS at week 12. These findings suggest that both baseline-MADRS score and depression severity at week 1 could be used as early predictors of depression severity at week 12.

Interestingly, while both dose and concentration were simultaneously evaluated using path analysis, only drug concentration, but not dose, was associated with clinical outcomes. As previously described in chapter 3, there was a statistically significant correlation between dose and concentration at both week 1 and week 12. Due to the collinearity of dose and concentration, these variables could not be evaluated simultaneously as potential independent predictors of clinical outcomes in the regression analysis. However, path analysis allows the simultaneous evaluation of direct and indirect effects on a dependent variable even when independent variables are correlated. Our results would suggest that dose has an indirect effect on MADRS at week 12 which is mediated through drug concentration. These findings suggest measurement of venlafaxine concentration, possibly through TDM program, may serve as a useful tool for optimization of the dosing regimen and improvement of outcomes in MDD patients.

Similar to the findings reported in chapter 4, there was a negative correlation between concentration at week 1 and MADRS at week 12. Since nearly all patients were on the same dose at week 1, variability in drug concentration is likely due to factors related to PK. For example, patients with lower drug clearance due impaired renal or hepatic function would be expected to have higher drug concentration and improved clinical outcomes. Our results demonstrate that patients with higher concentration at week 1 are associated with improved outcomes at week 12. These results suggest that drug concentration at week 1 may serve as an early predictor of treatment response in MDD patients on venlafaxine.

Also, similar to the findings reported in chapter 4, there was a positive correlation between concentration at week 12 and MADRS at week 12. Since patients were titrated to higher doses

from week 6 to week 12 based on clinical response, it is expected that patients with worse outcomes would receive higher doses and thus have higher drug concentration at week 12. Our results demonstrate that patients with worse outcomes were associated with higher drug concentration. Although drug concentration at week 12 may not serve as an early predictor of treatment response in MDD patients on venlafaxine, our findings suggest that drug concentration is a key mediator of clinical response with venlafaxine treatment.

In chapter 5, we evaluated the effect of dose/drug concentration on functional connectivity. We did not observe any association between these relationships. Using path analysis, we evaluated the effect of dose and drug concentration on functional connectivity in the brain simultaneously. We observed a positive correlation between drug concentration and functional connectivity. As described above, patients were titrated to higher doses from week 6 to week 12 based on clinical response, it is expected that patients with worse outcomes would receive higher doses and thus have higher drug concentration at week 12. Our results demonstrate that patients with worse outcomes were associated with higher drug concentration.

In addition, our results demonstrate that venlafaxine dose has an indirect effect on functional connectivity in the brain which is mediated through drug concentration at week 12. Although drug concentration at week 12 may not serve as an early predictor of treatment response in MDD patients on venlafaxine, our findings suggest that drug concentration is a key mediator of functional connectivity.

6.5 Conclusions

In summary, we used the path analysis approach to simultaneously evaluate the direct and indirect relationships between venlafaxine dose, drug concentration, changes in neuronal connectivity, and clinical outcomes in depressed patients. The path analysis approach revealed relationships which were previously not observed using regression methods including the indirect effect of dose on clinical outcomes which was mediated through drug concentration. These findings suggest that the efficacy and safety of venlafaxine treatment of patients with MDD may be optimized through dose titration based on measurement of drug concentration possibly thought a TDM program.

7.0 Conclusions and Future Directions

7.1 Conclusions

7.1.1 Summary of Research Goals

There are two main goals for this thesis. First, investigate the relationships between both serum BDNF levels and Val66Met polymorphism, and the development of MDD in HCV patients on INF-α therapy. The second goal is to investigate the relationships between venlafaxine dose, drug concentration, and both brain functional connectivity and clinical outcomes in MDD patients. Key findings from our research are summarized below.

7.1.2 Key Research Findings

In the first part of this thesis, we investigated the relationship between both serum BDNF levels and Val66Met polymorphism, and the development of MDD in HCV patients on INF-α therapy. We report that lower baseline BDNF levels was associated with higher depression symptoms during IFN-α treatment. Also, Met allele was associated with lower BDNF levels, however it was not associated with increased BDI-II. An exploratory comparison of individual BDI-II items indicated that the Met allele was associated with suicidal ideation, sadness, and worthlessness, but not neurovegetative symptoms. In addition, we observed that IFN-α therapy further decreased BDNF serum levels, but this decrease occurred regardless of depression development and of genotype. These findings support the hypothesis that increased BDNF

improves resiliency against developing inflammatory cytokine-associated depression, and specifically to a subset of symptoms.

Next, we investigated the relationship between venlafaxine dose and drug concentration in MDD patients. We observed a weak positive correlation between venlafaxine dose and drug concentration at an early time point and a strong positive correlation at late time point. In addition, we report that patients ≥ 65 years old and patients with lower BMI had significantly higher drug concentration. These findings suggest that the venlafaxine dose may not be a clinically relevant predictor of drug concentration, and possibly clinical effect, in the early stages of treatment. Moreover, demographic and patients related factors such as age and BMI should be taking into account during dose adjustment particularly in elderly patients taking venlafaxine treatment.

Then, we investigated the relationships between both venlafaxine dose and drug concentration, and clinical outcomes. Our results demonstrated an association between drug concentration, but not venlafaxine dose, and clinical outcomes (change in MADRS score). More specifically, higher drug concentration at week 1 was associated with improved clinical outcomes. Also, higher baseline-MADRS severity was associated with worse outcomes. Taken together, these studies suggest that venlafaxine dose might not be a good predictor of change in MADRS scores. Also, achieving a high drug concentration after initiation of treatment may lead to earlier onset of response and/or improved outcomes in depressed patients. In addition, it may be helpful for clinicians to account for pre-treatment depression severity when developing an antidepressant treatment regimen.

In addition, we investigated the relationships between both venlafaxine dose and drug concentration, and functional connectivity in the brain in MDD patients. We observed an association between improved clinical outcomes, and both decreased and increased in ΔrIFG-DMN and ΔrMTG-DMN functional connectivity, respectively. However, we did not observe an association between functional connectivity in our candidate brain regions and venlafaxine dose nor drug concentration in MDD patients. Taken together, these data suggest that alteration of DMN connectivity may impact clinical outcomes in MDD patients on antidepressant therapy. Also, it is possible that the effect of venlafaxine dose and drug concentration on clinical outcomes are not mediated through functional connectivity. Thus, additional studies are needed to further investigate the relationships between venlafaxine dose, drug concentration, and functional connectivity in the brain in depressed patients.

Finally, we developed a model to describe the relationship between venlafaxine dose, drug concentration, functional connectivity in the brain and clinical outcomes at each time point simultaneously using path analysis approach. Our results suggest that dose has an indirect effect on MADRS at week 12 which is mediated through drug concentration. These findings suggest that measurement of venlafaxine concentration, possibly through a TDM program, may serve as a useful tool for optimization of the dosing regimen and improvement of outcomes in MDD patients.

7.2 Future Directions

7.2.1 Drug Development Targeting Psychiatric and Neurologic Diseases

In the United States, the economic burden of depressive disorders is estimated to be more than $210 billion with direct costs, costs related to suicide, and workplace costs accounting for approximately 45%, 5%, and 50%, respectively.[158] Although there are substantial unmet medical needs for improving mental health and reducing healthcare costs, the development of drugs that target psychiatric and neurologic diseases presents some unique challenges which may increase the time, cost, and risk for approval.[158, 191] For example, there is an extended time for both drug development and FDA review for drugs targeting psychiatric and neurologic diseases, which further adds to the cost for drug development.[191] In addition, the overall success rate for the development of psychiatry and neurology drug candidates is 6.2% and 8.4%, respectively, which is lower than 9.6% value reported across all therapeutic classes of drugs.[192] There are several factors which likely contribute to the extended time and cost and low success rates for development of psychiatric and neurologic drugs including disease heterogeneity, target identification and validation, poor animal models, overcoming the blood-brain barrier obstacles, and high placebo response.[191] As a result, several large pharmaceutical companies shifted their drug development efforts away from neuroscience.[193]

7.2.2 Current Status of Drug Development for The Treatment of MDD

Current pharmacological treatments for MDD present many unique challenges including highly variable response rates, treatment-resistant depression, and commonly observed side effects

such as weight gain, sexual, and cardiovascular problems.[157, 158, 194] As a result, there are on-going research efforts to identify novel drugs and targets for the treatment of MDD[157, 194] with a recent focus on treatment-resistant depression[195]. Currently, there are several novel investigational molecules for the treatment of MDD in various phases of drug development.[157, 194, 195] These novel investigational molecules can be broadly classified based on their pharmacology into groups such as opioid receptor modulators, *N*-methyl-D-aspartate (NMDA) receptor modulators, ionotropic or metabotropic glutamate receptor modulators, serotonergic receptor agonists, neurotrophins, triple re-uptake inhibitors, and others. [157, 194, 195] In addition, a few products approved by the FDA for indications other than depression are currently being investigated in the treatment of MDD. For example, the psychotropic drug ziprasidone is currently in Phase II clinical trials for the treatment of MDD.[195] Likewise, the antibiotic drug minocycline and the immunosuppressive agents tocilizumab and sirukumab present novel therapeutic options currently being investigated in clinical trials for the treatment of MDD.[195] In summary, there are several novel investigational molecules that serve as promising drug candidates for the treatment of MDD.

7.2.3 BDNF As A Therapeutic Target

BDNF has multiple effects in the brain including the support and survival of existing neurons, growth and differentiation of new neurons, and role in synaptic transmission.[196] Also, preclinical, clinical, and postmortem studies have shown that reduced BDNF levels are associated with a psychiatric and neurodegenerative disorders.[197] Thus, BDNF and its receptors serve as potential therapeutic target for depression and neurodegenerative diseases. However, there are several challenges with the delivery of the BDNF protein as a pharmacotherapy for depression and neurodegenerative diseases. For example, the PK of the intact BDNF protein is undesirable for

drug development. Specially, BDNF has been shown to have poor oral bioavailability due to hydrolysis[198] and a short *in vivo* half-life (<10 minutes).[199] In addition, BDNF does not readily cross the blood-brain barrier due to its large size.[200] Moreover, infusion of BDNF protein into the cerebrospinal fluid (CSF) is invasive and presents many clinical risks.[197] These challenges have delayed the development of BDNF as a potential pharmacotherapy for depression and neurodegenerative diseases.

7.2.4 BDNF Delivery Strategies

On the other hand, pre-clinical research targeting BDNF as a therapeutic modality continues to progress at a rapid speed. Much of the recent advances in developing BDNF as a drug candidate have been focused on improving the drug delivery strategies. Geral et al. provides an excellent review on emerging strategies for delivery of BDNF and other neurotrophins in pre-clinical models along with a few clinical studies.[198] These emerging approaches include administration of recombinant BDNF by direct injection, osmotic pump, or other drug delivery methods, cell- and viral-mediated BDNF delivery, sustained-release technologies using synthetic and naturally occurring polymer systems or lipid-based formulations containing BDNF, increasing BDNF levels through diet and exercise, and peptidomimetics, small molecule mimetics, and prodrugs which activate TrkB receptors or modulate receptor activity. To our knowledge, none of these approaches has resulted in an FDA approved and marketed product targeting BDNF as a pharmacotherapy for depression or other neurodegenerative diseases.

7.2.5 BDNF Clinical Trials in MDD Patients

There are numerous clinical trials which investigated the role of BDNF in the pathophysiology of MDD or include the measurement of BDNF as a biological marker or predictor of response to treatment in MDD patients.[201] However, there are no on-going or completed clinical trials in MDD patients which investigate the emerging drug delivery strategies used in animal or *in vitro* models for the direct delivery of BDNF or BDNF mimetics. In a phase III clinical trial involving amyotrophic lateral sclerosis (ALS) patients, recombinant methionyl human BDNF was delivered via subcutaneous and intrathecal injection, but the study failed to show benefit of BDNF treatment for the primary end points.[202] Collectively, these observations suggest that drug development targeting the BDNF pathway has demonstrated very limited success in clinical trials.

7.2.6 Future Directions for Drug Development Targeting BDNF

As previously stated, there are several challenges with the direct delivery of the BDNF protein as a potential treatment for MDD patients. In my opinion, the development of peptidomimetics, small molecule mimetics, and prodrugs which activate TrkB receptor offer several potential advantages over other emerging approaches to target TrkB-BDNF signaling pathway in the treatment of MDD. For example, certain prodrugs in pre-clinical development have been shown to pass the blood-brain barrier and reduce depression- and anxiety-related behaviors in rats.[197] In addition, small molecule agonists of the TrkB receptor are more likely to exhibit pharmacokinetic properties which are more desirable for drug development including increased bioavailability, half-life, and distribution in the brain when compared to BDNF protein. Also, it may be easier to develop an oral formulation of a small molecule than a protein during drug

development. It is well established that oral drug delivery is the most preferred and convenient route of drug administration due to high patient compliance, non-invasiveness, cost-effectiveness, flexibility in the design of the dosage form, and ease of production.[203] As such, the development of an orally-administered small molecule agonist of the TrkB receptor may serve as a promising strategy for the treatment of depression. However, it is important to note that pre-clinical studies reported that overexpression of BDNF in mice was associated with side effects in the CNS possibly through hyperactivation of TrkB receptor. Therefore, further studies are needed to investigate not only the PK, but also the safety and efficacy, of small molecule agonists of the TrkB receptor in the treatment of MDD and other neurological disorders.

7.2.7 Future of Drug Development for The Treatment of MDD

The future of drug development for the treatment of MDD will likely focus on drug candidates which demonstrate a faster onset of action, improved efficacy, and/or reduced side effects. Recent clinical studies have demonstrated that ketamine is a promising agent for managing treatment-resistant depression.[195] In addition, ketamine and other NMDA receptor antagonists have been shown to significantly reduce the time to clinical response in MDD patients, which may be especially important for patients with suicidal ideationt.[157, 194, 195] However, side effects are commonly observed with ketamine treatment and the potential for abuse and addiction continue to present a major concern.[157, 194, 195] Other investigational molecules in development, such as the glycine site partial agonist AV-101 and the glycine receptor antagonist rapastinel (GLYX-13), have reported rapid and persistent antidepressant effects without the psychotomimetic effects observed with ketamine treatment.[157, 194, 195] In my opinion, rapastinel is the most promising drug candidate currently in development for the treatment of MDD because it passes the blood-brain

barrier, demonstrates rapid and long-lasting antidepressant properties, enhances cognitive abilities, and lacks psychotomimetic side effects.[157, 194, 195] Currently, there are 2 completed and 8 active clinical trials investigating rapastinel in the treatment of MDD and most of these studies are in Phase III of development.[201]

Recently, the FDA published a new draft guidance for industry titled "Major Depressive Disorder: Developing Drugs for Treatment (June 2018)" to assist sponsors in the clinical development of drugs for monotherapeutic, combination, and adjunctive treatment of MDD.[204] This guidance is intended to serve as a focus for continuous discussions among the Division of Psychiatry Products at the FDA, pharmaceutical companies, academia, and the public. It is expected that this new draft guidance will encourage the development of new therapies for MDD by clarifying the regulatory requirements and possibly reducing the associated costs and risks.

7.2.8 Population PK for Optimization of Venlafaxine Pharmacotherapy in MDD

As previously described in Chapter 3 and Chapter 4, venlafaxine exhibits highly variable drug concentration and the drug exposure-response relationships are not well studied. Therefore, a better understanding of the impact of interindividual variability in venlafaxine drug concentration on clinical outcomes may improve the treatment efficacy, reduce the treatment time required to achieve a clinically significant response, and potentially reduce the number and severity of adverse events associated with treatment. In my opinion, population PK modeling and simulation may be useful to identify and quantify sources of variability in venlafaxine PK and then to predict PK-PD relationships for individual patients or subgroups.[205-207] In this manner, the dosing regimen for each patient could be individualized based on clinical and demographic factors such as age, weight, comorbidities, and concomitant medications. The benefits of population PK modeling are well

established[208-210] and its use in drug development is recommended by the FDA to help identify differences in drug safety and efficacy among population subgroups[211]. Nevertheless, there are only a few published studies of population PK analysis of venlafaxine in the literature.[29, 212, 213] Therefore, in my opinion, population PK may be a useful tool for the optimization of venlafaxine pharmacotherapy in MDD patients.

8.0 Limitations

The limitations for the first part of this thesis are summarized below.

We investigated the effect of a single inflammatory cytokine (interferon-α) and we did not control for concomitant medications. We evaluated the effect of Val66Met but other BDNF polymorphisms may affect BDNF levels. Loss of follow-up overtime (patients dropped out from the study after they develop depression). The correlation between peripheral and central BDNF levels in human is unknown. We did not control for demographics in our analysis. The generalization to other types of depression is minimized by selection of resilient subjects who were not depressed despite their chronic hepatitis C infection.

The limitations for the second part of this thesis are summarized below.

The study had a relatively small sample size of 54 patients. We investigated the effect of a single antidepressant treatment (venlafaxine) and did not control for concomitant medications and non-pharmacological treatments. We did not evaluate any genetic or biological biomarkers and their impact on venlafaxine PK and/or PD. In addition, this study did not include a placebo group and therefore, it is difficult to conclude that the changes in the depressive symptoms is due to either venlafaxine treatment or other factors such as patient expectations and attention from clinicians.

Also, it is possible that earlier response to treatment could be explained by having lower depressive score and not due to treatment effect. Our study used one form of the depression scale (MADRS) and may not be generalized to other depression measurement scales. Furthermore, this

study included sparse concentrations sampling. We attempted to perform modeling and simulation using non-linear mixed effect model (NONMEM) to predict venlafaxine concentration over time, but the predicted PK parameters were highly variable and lacked precision due to limited data, especially during the absorption phase. Since the venlafaxine product label recommends the titration of the dose based on clinical effect, comparisons between studies are difficult due the variable dosing regimens and duration of therapy. The correlation between drug concentration in the plasma and the site of action is unknown. We limited our analyses to two ROIs that represented core nodes of the default mode and executive control, however, each of these networks has multiple nodes that we did not explore. Finally, differences at the final time point between responders/non-responders may be important in predicting changes in depression symptoms, however, group differences do not necessarily give the ability to distinguish individual subjects.

Appendix A Montgomery-Asberg Depression Rating Scale (MADRS) Form

STRUCTURED INTERVIEW GUIDE FOR THE MONTGOMERY AND ASBERG DEPRESSION RATING SCALE (SIGMA)

ID_ _ _ _ _ _ _	DATE _ _/_ _/_ _ _ _ m m d d y y	CLINICIAN NUMBER _ _ _ _ _ _

Janet B.W. Williams, D.S.W. and Kenneth A. Kobak, Ph.D.

INTERVIEWER: The questions in bold for each item should be asked exactly as written. Often these questions will elicit enough information about the severity and frequency of a symptom for you to rate the item with confidence. Follow-up questions are provided, however, for use when further exploration or additional clarification of symptoms is necessary. The specified questions should be asked until you have enough information to rate the item confidently. In some cases, you may also have to add your own follow-up questions to obtain necessary information. Note that questions in parentheses are optional, i.e., if information is unknown.

NOTES:

Time period. The ratings should be based on the patient's condition in the past week.

Change from baseline. In general, a symptom is rated as present only when it reflects a change from before the depression began. The interviewer should try to identify a 2-month period of non-depressed functioning and use this as a reference point. In some cases, such as when the patient has Dysthymia, the referent should be to the last time they felt OK (i.e., not depressed or high) for at least a few weeks.

*Remember: If symptom is endorsed by patient, to always consider **duration, frequency, and impairment** in functioning!

This interview guide is based on the Montgomery and Asberg Depression Rating Scale (MADRS) (Montgomery SA, Asberg M: A new depression scale designed to be sensitive to change. Brit J Psychiat 134:382-389, 1979). The scale itself has been retained in its original form, except for reversing the order of the first two items. This guide adds interview questions to aid in the assessment and application of the MADRS. Previous versions of this guide appeared in 1988, 1992, 1996, and 2005.

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Sigma v.1, 2007

STRUCTURED INTERVIEW GUIDE FOR THE MONTGOMERY AND ASBERG DEPRESSION RATING SCALE (SIGMA)

PT'S INITIALS: _____ PT'S ID: _____ **TIME BEGAN SIGMA: _____**

INTERVIEWER: _____ **DATE: __ __ __ __ __ __**

OVERVIEW: I'd like to ask you some questions about the past week. How have you been feeling since last (DAY OF WEEK)? IF OUTPATIENT: Have you been working? (What kind of work do you do?) IF NOT: Why not?

RATING BASED ON OBSERVATION DURING INTERVIEW AND THE FOLLOWING QUESTIONS.

In the past week, do you think you have looked sad or depressed to other people? Did anyone say you looked sad or down?

How about when you've looked in the mirror? Did you look gloomy or depressed?

IF YES: How sad or depressed do you think you have looked? How much of the time over the past week do you think you have looked depressed or down?

IF APPEARANCE WAS DEPRESSED IN PAST WEEK: Have you been able to laugh or smile at all during the past week? IF YES: How hard has it been for you to laugh or smile, even if you weren't feeling happy inside?

1. APPARENT SADNESS. Representing despondency, gloom and despair. (More than just ordinary transient low spirits) reflected in speech, facial expressions, and posture. Rate by depth and inability to brighten up.

0 – No sadness
Appears happy during the interview.

1 – *Appears slightly down only momentarily during the interview.*

2 - Looks dispirited but does brighten up without difficulty.
Appears down during the interview, but brightens up from time to time (e.g., laughing at a joke or amusing story; smiles when shaking hands w/ interviewer, etc.)

3 - *Appears sad during interview and only responds vaguely and with difficulty to positive interactions within the interview.*

4 - Appears sad and unhappy most of the time.
Appears sad and unhappy throughout interview. This is reflected both verbally and non-verbally.

5 – *Appears more severely down than a 4. (e.g., weeps throughout interview, etc.)*

6 – Looks miserable all the time. Extremely despondent.
As reflected in speech, facial expression, and posture.

In the last week, have you been feeling sad or unhappy? (Depressed at all?) IF YES: Can you describe what this has been like for you? (IF UNKNOWN: How bad has that been?)

IF DEPRESSED: Does the feeling lift at all if something good happens? How much does your mood lift? Does the feeling ever go away completely? (What things have made you feel better?)

How often did you feel (depressed/OWN EQUIVALENT) this past week? (IF UNKNOWN: How many days this week did you feel that way? How much of each day?)

In the past week, how have you been feeling about the future? (Have you been discouraged or pessimistic?) What have your thoughts been? How (discouraged or pessimistic) have you been? How often have you felt that way? Do you think things will ever get better for you?

IF ACKNOWLEDGES DEPRESSED MOOD, TO GET CONTEXT ASK: How long have you been feeling this way?

2. REPORTED SADNESS. Representing reports of depressed mood, regardless of whether it is reflected in appearance or not. Includes low spirits, despondency or the feeling of being beyond help and without hope. Rate according to intensity, duration, and the extent to which the mood is reported to be influenced by events. **Based strictly on patient report.**

0 - Occasional sadness in keeping with the circumstances.

1 – *Occasional reports of out-of-the-blue sadness.*

2 - Sad or low but brightens up without difficulty.

3 – *More frequent sadness than #2. Mood somewhat brightens to normal level, but only with difficulty, when pleasant events occur.*

4 - Pervasive feelings of sadness or gloominess. The mood is still influenced by external circumstances. *Mood never fully reaches "normal" brightening level, and is very brief in duration.*

5 – *Near continuous sadness. External circumstances no longer influence mood.*

6 – Continuous or unvarying sadness, misery, or despondency.

Have you felt tense or edgy in the last week? Have you felt anxious or nervous?
IF YES: Can you describe what that has been like for you? How bad has that gotten? (Have you felt panicky?)

What about feeling fearful that something bad is about to happen?

How hard has it been to control these feelings? (What has it taken to help you feel calmer? Has anything worked to calm you down?)

How much of the time have you felt this way over the past week?

3. INNER TENSION. Representing feelings of ill-defined discomfort, edginess, inner turmoil, mental tension mounting to either panic, dread, or anguish. Rate according to intensity, frequency, duration and the extent of reassurance called for.

0 - Placid. Only fleeting inner tension.
Endorses a sense of inner calm, w/ only brief moments of tension or edginess.

1 - *Reports doubtful or trivial symptoms of inner turmoil, edginess, or tension, which are rare and cause little to no distress.*

2 - Occasional feelings of edginess and ill-defined discomfort.
Endorses several occasions of tension/edginess that cause some discomfort. Distress is manageable, and does not impair functioning.

3 – *Reports frequent incidents of tension/edginess which cause moderate distress, and some impairment in functioning.*

4 – Continuous feelings of inner tension or intermittent panic which the patient can master with some difficulty. *A nearly constant sense of anxiety and inner tension. Able to deal with symptoms, but they cause significant impairment in functioning.*

5 – *Reports an inability to deal with these symptoms.*

6 – Unrelenting dread or anguish. Overwhelming panic.
Severely impaired to a greater degree than a 5. Symptoms are constant, overwhelming and severely debilitating.

How has your sleeping been in the last week? (How many hours have you been sleeping, compared to usual?)

Have you had trouble falling asleep? (How long has it been taking you to fall asleep this past week?)

Have you been able to stay asleep through the night? (Have you been waking up at all in the middle of the night? How long does it take you to fall back asleep?)

Has your sleeping been restless or disturbed?

4. REDUCED SLEEP. Representing the experience of reduced **duration or depth** of sleep compared to the subject's own normal pattern when well.

0 - Sleeps as usual.

1 - *Reports minimal sleep disruption (e.g., one night w/ mildly disturbed sleep)*

2 - Slight difficulty dropping off to sleep or slightly reduced, light, or fitful sleep. *Reports slight sleep difficulties on several occasions during the week.*

3 – *Reports moderate sleep difficulties on several evenings during the week (e.g., 1-1/2 hour delay in falling asleep on several occasions; and/or waking 1-1 ½ hours earlier than usual on several mornings).*

4 – Sleep reduced or broken by at least two hours. *Reports sleep difficulties resulting in a reduction or disturbance of sleep at least 2 hours.*

5 – *Reports nightly sleep difficulties resulting in a reduction or disturbance of sleep by greater than 2 hours.*

6 – Less than two or three hours sleep. *Severe, nightly sleep difficulties, resulting in significantly reduced amounts of sleep (e.g., total sleep time less than 2-3 hours).*

How has your appetite been this past week? (What about compared to your usual appetite?)

Have you been less interested in food? (How much less?)

Does food taste as good as usual? IF LESS: How much less?

Have you had to force yourself to eat?

Have other people had to urge you to eat?

5. REDUCED APPETITE. Representing the feeling of a loss of appetite compared with when well. Rate by loss of desire for food or the need to force oneself to eat.

0 - Normal or increased appetite.

1 – *Reports a doubtful or trivial decrease in appetite.*

2 - Slightly reduced appetite. *Reports mild decrease in appetite; the patient eats without encouragement by others, food intake is about normal.*

3 – *Reports a moderate decrease in appetite, with a clearly reduced food intake.*

4 – No appetite. Food is tasteless. *Endorses a severe loss of appetite; food intake is markedly decreased.*

5 – *Patient is barely consuming any food.*

6 – Needs persuasion to eat at all. *Loss of appetite is obvious and complete. Patient would stop eating entirely if not encouraged by others.*

Have you had trouble concentrating or collecting your thoughts in the past week? (How about at home or at work?) IF YES: Can you give me some examples? (Have you been able to concentrate on reading a newspaper or magazine? Do you need to read things over and over again?)

How often has that happened in the past week? Has this caused any problems for you? IF YES: Can you give me some examples?

Has your trouble concentrating been so bad at any time in the past week that it has been difficult to follow a conversation? (IF YES: How bad has that been? How often has that happened this past week?)

NOTE: ALSO CONSIDER BEHAVIOR DURING INTERVIEW.

6. CONCENTRATION DIFFICULTIES. Representing difficulties in collecting one's thoughts mounting to incapacitating lack of concentration. Rate according to intensity, frequency, and degree of incapacity produced.

0 - No difficulties in concentration.

1 - *Extremely mild, possibly doubtful difficulties in concentrating.*

2 - Occasional difficulties in collecting one's thoughts. *Difficulties are infrequent and do not interfere with functioning.*

3 – *Reports frequent difficulties collecting one's thoughts, that do not significantly interfere with functioning.*

4 – Difficulties in concentrating and sustaining thought which reduces ability to read or hold a conversation. *Reports a decreased ability to function due to concentration difficulties.*

5 – *Reports pervasive difficulties and significantly impaired functioning.*

6 – Unable to read or converse without great difficulty. *The patient is barely able to read or communicate, making the interview extremely difficult or impossible to complete.*

Have you had any trouble getting started at things in the past week? IF YES: What things?

Have you had to push yourself to do things? IF YES: What things? How hard have you had to push yourself? Are you OK once you get started or is it still more of an effort to get something done? What about getting started at simple routine everyday things (like getting dressed)?

Have you done everyday things more slowly than usual? (Have you been sluggish?) IF YES: Like what, for example? How bad has that been?

7. LASSITUDE. Representing a difficulty getting started, or slowness initiating and performing everyday activities.

0 - Hardly any difficulty in getting started. No sluggishness.

1 – *Reports a slight difficulty in starting one or two activities in the past week. This difficulty does* ***not*** *extend to multiple areas and does not interfere with the ability to function.*

2 - Difficulties in starting activities. *Clearly endorses difficulty, however, once activities are started the patient is able to carry them out.*

3 – *Patient has pervasive difficulties starting activities in* ***many*** *different areas.*

4 – Difficulties in simple routine activities which are carried out with effort. *Difficulties starting even simple routines (e.g., basic chores, grooming, etc.). Once these routines are started, patient has difficulty completing them.*

5 – *Pervasive difficulties in multiple areas of functioning. Functioining is severely affected.*

6 – Complete lassitude. Unable to do anything without help. *Unable to initiate or complete any tasks without assistance.*

Have you been less interested in things around you, or in activities you used to enjoy? IF YES: What things? How bad has that been? How much less interested in (those things) are you now compared to before?

Have you been less able to enjoy the things you usually enjoy?

Has there been any change in your ability to feel emotions? (Do you feel things less intensely than you used to, things like anger, grief, pleasure?) IF YES: Can you tell me more about that? (IF UNKNOWN: Are you able to feel any emotions at all?)

How do you feel toward your family and friends? Is that different from usual? IF REDUCED: Do you feel less than you used to towards them?

8. INABILITY TO FEEL. Representing the subjective experience of reduced interest in the surroundings, or activities that normally give pleasure. The ability to react with adequate emotion to circumstances or people is reduced.

0 - Normal interest in the surroundings and in other people.

1 – *Reports slightly reduced pleasure or interest in people or certain activities. Most people and activities are still as interesting and enjoyable.*

2 - Reduced ability to enjoy usual interests. *Reports getting clearly less pleasure or enjoyment from some activities.*

3 – *A marked loss of pleasure in many activities.*

4 – Loss of interest in the surroundings. Loss of feelings for friends and acquaintances. *Patient has clearly lost interest in most activities, and/or reports a loss of emotion or feelings towards some people.*

5 – *Patient has lost feelings and interest for* ***most*** *people and activities.*

6 – The experience of being emotionally paralyzed, inability to feel anger, grief, or pleasure, and a complete or even painful failure to feel for close relatives and friends.

Have you been putting yourself down, or feeling that you're a failure in some way, over the past week? (Have you been blaming yourself for things that you've done, or not done?) IF YES: What have your thoughts been? How often have you felt that way?

Have you been feeling guilty about anything in the past week? What about feeling as if you have done something bad or sinful? IF YES: What have your thoughts been? How often have you felt that way?

ALSO CONSIDER RESPONSES TO QUESTIONS ABOUT PESSIMISM FROM ITEM #1.

9. PESSIMISTIC THOUGHTS. Representing thoughts of guilt, inferiority, self-reproach, sinfulness, remorse, and ruin.

0 - No pessimistic thoughts. *Reports no pessimism OR self-critisim.*

1 – *Reports feeling slightly self-critical or mildly pessimistic about the future.*

2 - Fluctuating ideas of failure, self-reproach, or self-depreciation. *Pt is clearly self-critical on occasion. Such feelings are not persistent.*

3 – *Feels self-critical on more days than not, and/or is clearly discouraged about the future.*

4 – Persistent self-accusations, or definite but still rational ideas of guilt or sin. Increasingly pessimistic about the future. *Endorses constant feelings of self-critism or guilt, and/or is increasingly pessimistic and severely discouraged about the future.*

5 – *Patient's negative thoughts about the self or future border on "delusional," but do not quite reach that level of severity.*

6 – Delusions of ruin, remorse, or unredeemable sin. Self-accusations which are absurd and unshakable.

This past week, have you felt like life isn't worth living? IF YES: Tell me about that. IF NO: What about feeling like you're tired of living?

This week, have you thought that you would be better off dead? IF YES: Tell me about that.

Have you had thoughts of hurting or even killing yourself this past week? IF YES: What have you thought about? How often have you had these thoughts? How long have they lasted? Have you actually made plans? IF YES: What are these plans? Have you made any preparations to carry out these plans? (Have you told anyone about it?)

10. SUICIDAL THOUGHTS. Representing the feeling that life is not worth living, that a natural death would be welcome, suicidal thoughts, and preparation for suicide. Suicidal attempts should not in themselves influence this rating.

0 - Enjoys life or takes it as it comes. *Denies suicidal ideation or a feeling that life is not worth living.*

1 – *Endorses a vague, transient sense that life is not worth living, without any specific wishes to die or thoughts of self-harm.*

2 - Weary of life. Only fleeting suicidal thoughts. *Reports a sense that life is not worth living, and has had some passing thoughts of suicide. There is no specific plan or intent for self-harm.*

3 – *Endorses frequent thoughts of self-harm and a strong sense that life is not worth living, without any specific plan or intent to die. Suicide is not considered an option.*

4 - Probably better off dead. Suicidal thoughts are common, and suicide is considered as a possible solution, but without specific plans or intention. *Actively considering suicide as an option, without any specific plans or intentions..*

5 – *Patient has a specific plan for suicide and a strong wish to die, without having made a firm decision to carry it out.*

6 – Explicit plans for suicide when there is an opportunity. Active preparations for suicide. *Patient would kill himself/herself if they had the chance.*

TOTAL MADRS SCALE SCORE: _____ _____

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Appendix B Cumulative Illness Rating Scale-Geriatric (GIRS-G) Form

Scoring Sheet

CUMULATIVE ILLNESS RATING SCALE FOR GERIATRICS (CIRS-G)

Miller, Paradis, and Reynolds 1991

PATIENT_____AGE_____

RATER_____DATE_____

Instructions: Please refer to the CIRS-G manual. Write brief descriptions of the medical problem(s) that justified the endorsed score on the line following each item. (Use reverse side for more writing space.)

RATING STRATEGY

0- No problem
1- Current mild problem or past significant problem
2- Moderate disability or morbidity/requires first line therapy
3- Severe/ constant significant disability/ uncontrollable chronic problems
4- Extremely severe/ immediate treatment required/ end organ failure/ severe impairment in function

	SCORE
HEART	_____
VASCULAR	_____
HEMATOPOIETIC	_____
RESPIRATORY	_____
EYES, EARS, NOSE, THROAT AND LARYNX	_____
UPPER GI	_____
LOWER GI	_____
LIVER	_____
RENAL	_____
GENITOURINARY	_____
MUSCLOSKELETAL/INTEGUMENT	_____
NEUROLOGICAL	_____
ENDOCRINE/METABOLIC AND BREAST	_____
PSYCHIATRIC ILLNESS	_____

TOTAL NUMBER OF CATEGORIES ENDORSED	_____
TOTAL SCORE	_____
Severity index: (total score/total number of categories endorsed)	_____
Number of categories at level 3 severity	_____
Number of categories at level 4 severity	_____

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