Deanship of Graduate Studies

Al-Quds University

Hematological and Immunological Markers of Response to Selective Serotonin Reuptake Inhibitors in Patients with Major Depressive Disorder

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Thesis Approval

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Jerusalem – Palestine

1439 – 2017

Dedication

To my first inspiration,

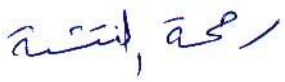
who held my hands in everyway throughout my life,

who gave me everything and who mean to me everything,

to my beloved parents, I dedicate my thesis.

Declaration

I certify that this thesis submitted for the degree of master, is the result of my own research, except where otherwise acknowledged, and that this study has not been submitted for a higher degree to any other university or institution.

Signed: 

Rahmeh Yousuf Saeed Natsheh

Date: 23/12/2017

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Abstract

Major depressive disorder (MDD) is a psychiatric disorder that is characterized by long-lasting sadness, depressed mood and loss of pleasure in life activities. It is the most prevalent mental health problem in the West Bank, Palestine, affecting around 30% of Palestinians. Recent studies argue that MDD can result from disruption to particular hematological and immunological pathways. Only 30% of patients with MDD respond to pharmacological modalities for treating MDD, the most commonly used of which are selective serotonin reuptake inhibitors (SSRIs). Unfortunately, there are no tools that can help predict in advance which MDD patient will, or will not respond to SSRIs. The aim of this study is to investigate immunological and hematological markers that have the potential to *a priori* predict response to SSRI antidepressants in patients with MDD to provide the field of psychiatry with valuable tools to select the appropriate treatment for the appropriate patient.

We recruited 8 medication-naïve patients with MDD from different psychiatric clinics in the West Bank, Palestine. These patients never received any form of treatment. Further, we tested 8 matched healthy control subjects. Patients with MDD were tested twice; (1) at baseline, upon diagnosis with MDD, and (2) 4-6 weeks after receiving SSRI treatment (estimated time for clinical improvement). Patients were assigned to two groups according to their response to SSRI treatment: (1) SSRI-responders and (2) SSRI-non-responders. Healthy subjects were also tested twice, 4-6 weeks apart. A peripheral blood sample was collected using an EDTA tube (10 mL) from each subject per session to investigate the levels of the following cytokines: IL-2, IL-6, IL-10, IFN-γ, MIF as well as for CRP quantification using ELISA. Further, we prepared blood films manually and examined them to obtain a differential count of the white blood cells.

Our results showed that SSRI-responders and SSRI non-responders show trending differences at baseline in both cytokine levels and cell counts. In particular, SSRI-responders show higher levels of both pro-inflammatory and anti-inflammatory cytokines. Conversely, non-responders showed relatively lower levels of pro-inflammatory and anti-inflammatory cytokines. Hematological markers differentiated patients with MDD from healthy controls with higher levels of lymphocytes and lower levels of neutrophils and eosinophils. After treatment, SSRI-

responders, but not non-responders, showed a relative decrease in pro-inflammatory cytokines and lymphocytes.

These results could present an initial signal for potential *a priori* molecular markers of response to SSRI in patients with MDD. With sufficient recruitment of patients, we anticipate that statistical classification models can produce significant differences between responders and non-responders using the immunological and hematological markers under investigation in this study.

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Definitions

Major depressive disorder: is a psychiatric disorder that is characterized by long-lasting sadness, depressed mood and loss of pleasure in life activities and it is the most prevalent mental health problem in the West Bank, Palestine, affecting around 30% of Palestinians

Selective serotonin reuptake inhibitor: is the most commonly used pharmacological agents to treat MDD, which increases the synaptic availability of serotonin.

Abbreviations

CRP: C-Reactive Protein

HPA Axis: Hypothalamic–Pituitary–Adrenal Axis

HC: Healthy Control

IFN: Interferon

IFN-α: Interferon Alpha

IFN-γ: Interferon Gamma

IL-1: Interleukin One

IL-1β: Interleukin One Beta

IL-2: Interleukin Two

IL-4: Interleukin Four

IL-6: Interleukin Six

IL-10: Interleukin Ten

MDD: Major Depressive Disorder

MIF: Macrophage Migration Inhibitory Factor

SSRI: Selective Serotonin Reuptake Inhibitor

TNF-α: Tumor Necrosis Factor alpha

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Major Depressive Disorder (MDD)

1.1.1 Clinical significance and prevalence of MDD in Palestine

MDD is a psychiatric disorder that is characterized by long-lasting sadness, depressed mood and loss of pleasure in life activities (Belmaker and Agam 2008). MDD is the most prevalent mental health problem in the West Bank, Palestine, affecting around 30% of Palestinians (Canetti, Galea et al. 2010, Madianos, Sarhan et al. 2012) . The pathophysiology of MDD is0 not very well understood. MDD can result from disruption to the immunological pathways. Several studies found that MDD patients exhibit variations in the levels of cytokines; immunological molecules that drive and coordinate immune response (Myint, Leonard et al. 2005, Lee and Kim 2006) Further, some studies showed that cytokine treatment in patients with malignancy and hepatic disorders may precipitate MDD-like symptoms (Horikawa, Yamazaki et al. 2003). On the other hand, Schiepers et al. as well showed that external or internal stress induces cytokine imbalance that plays a role in MDD symptom expression in vulnerable individuals (Schiepers, Wichers et al. 2005). Taken together, these findings suggest that MDD could potentially have an immune component.

1.1.2 The cytokine hypothesis of MDD

Several studies have shown that MDD patients exhibit variations in immune response. The immune response is mediated by cytokines that are messenger molecules produced mainly by monocytes, lymphocytes and activated glial cells. Cytokines include five different groups; interleukins, chemokines, tumor necrosis factors, interferons, and transforming growth factors (Jeon and Kim 2016). According to their activity; cytokines are divided into two groups: (1) Pro-inflammatory cytokines that are directly or indirectly involved in the inflammatory processes, such as interleukin one (IL-1), interleukin six (IL-6), and interferon gamma (IFN-γ), and (2) Anti-inflammatory cytokines, such as interleukin four (IL-4), interleukin ten (IL-10), that suppress the immune response by counteracting cellular activation as well as the

production of pro-inflammatory cytokines (Schiepers, Wichers et al. 2005). Therefore, pro-inflammatory cytokines and anti-inflammatory cytokines interact to maintain balance in the immune response. The production of the pro-inflammatory cytokines by macrophages or monocytes is determined by the immune activity (Jeon and Kim 2016).

One of the most widely accepted theories of the pathophysiology of MDD is the **cytokine hypothesis of MDD.** It proposes that the observed increased levels of pro-inflammatory cytokines, IL-1 and interleukin-2 (IL-2) in MDD patients correlate with the severity of MDD (Smith 1991). For example, Hauser et al. found that 33.3% of patients with hepatitis met the diagnostic criteria for MDD following initiation of interferon therapy (Hauser, Khosla et al. 2002). Further, some cognitive characteristics of MDD are similar to the symptoms of cytokine-induced sickness and range from subtle attentional deficits and memory impairments to delirium and psychosis. These impairments are also known to be associated with cytokine treatment of malignancies and chronic infections, such as interferon alpha (IFN-α) and IL-2 (Meyers 1999, Schiepers, Wichers et al. 2005).

1.1.3 Immunological changes in MDD

Increased levels of pro-inflammatory cytokines, such as IL-1, IL-2 and IL-6, by activated macrophages and IFN-γ by activated T-cells are observed in MDD patients (Myint, Leonard et al. 2005, Lee and Kim 2006). Musil et al. showed significantly elevated serum levels of the pro-inflammatory cytokine macrophage migration inhibitory factor (MIF) in patients with MDD (Musil, Schwarz et al. (2011)). However, there was no known mechanism of how MIF elicits its function on MDD (Leyton-Jaimes, Kahn et al. 2017). MIF blood levels were decreased by antidepressants in MDD patients as Cattaneo et al. showed (Cattaneo, Gennarelli et al. (2013)). Pro-inflammatory cytokines, such as IL-2, IL-6 and IFN-γ, may precipitate hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis by disturbing the negative feedback inhibition of circulating corticosteroids on the HPA axis (Sapolsky, Rivier et al. 1987, Harbuz, Stephanou et al. 1992, Pauli, Linthorst et al. 1998, Jeon and Kim 2016). Activation of the HPA axis increases cortisol secretion from the adrenal cortex, which is inversely related to the production of pro-inflammatory cytokines leading to a surge in their plasma concentrations (Watkins, Nguyen et al. 1999). Aside from the impaired inhibitory feedback of cortisol on its own production, the inhibitory feedback mechanism of cytokine

levels against increased levels of cortisol is also impaired in patients with MDD. These impairments may be directly related to the etiology of MDD (Jeon and Kim 2016).

Studies have shown that some patients who are treated with interferon fulfill the diagnostic criteria for MDD two months after the beginning of their treatment (Horikawa, Yamazaki et al. 2003). Another study showed that the increased levels of IL-6 and IFN-α are significantly associated with the severity of MDD symptoms (Capuron, Gumnick et al. 2002). These findings support the cytokine hypothesis of MDD which postulates that the severity of MDD correlates with the increased levels of pro-inflammatory cytokines, like IL-1 and IL-2 (Maes, Meltzer et al. 1995).

1.1.4 Hematological changes in MDD

Heightened immune activation in MDD includes an increasing number of circulating lymphocytes and phagocytic cells (Maes, Meltzer et al. 1995). Evidence suggests that MDD has been reliably associated with an absolute leukocytosis, a relative reduction in T-cell populations and low lymphocyte proliferative response to mitogen. Additionally, both MDD and acute/chronic stressors have been reliably related to reduced natural killer-cell function as well as increased CD4/CD8 cell ratios (Zorrilla, Luborsky et al. 2001, Tuglu, Kara et al. 2003). Moreover, Tuglu et al. showed that the leukocyte count of MDD patients decreased after antidepressant treatment (Tuglu, Kara et al. 2003).

1.2 Selective Serotonin Reuptake Inhibitors (SSRIs)

1.2.1 Definition

The monoamine hypothesis proposes that MDD results from decreased extracellular monoamines availability, especially serotonin (5-HT) and noradrenaline (Charney 1998). Selective serotonin reuptake inhibitors (SSRI), the most commonly used pharmacological agents to treat MDD, increases the synaptic availability of serotonin (Svenningsson, Kim et al. 2013).

Serotonin, which is a neuromodulator, plays a regulatory role in peripheral cytokine production through regulation of cortisol level. Cortisol secretion from the adrenal cortex increases by HPA axis activation, which is inversely related to the production of pro-

inflammatory cytokines, such as interleukin one beta (IL-1β) (Watkins, Nguyen et al. 1999). In MDD, the inhibitory mechanism that regulates cortisol and cytokine secretion against increased levels of cortisol is impaired (Jeon and Kim 2016). Serotonin inhibits the secretion of corticotrophin-releasing-hormone in the hypothalamus which ultimately decreases cortisol secretion. Further, inhibition of adrenocorticotropic hormone release in the pituitary also results in reduction of cortisol production. Thus, SSRI can restore the negative feedback of cortisol that occurs in MDD and normalize HPA axis function by increasing serotonin availability.

SSRI can also regulate the immune cell activity by acting as serotonin antagonists on the immune cells. Macrophages have a serotonin uptake system that is similar to the system in platelets; however, T- lymphocytes express serotonin receptors (5-HT1A and 5HT2A/2C) and high-affinity serotonin transporters. By blocking these receptors, and thus causing deficiencies in intracellular serotonin storage and increasing extracellular serotonin, SSRI can have a negative immuno-regulatory role in the immune cell activity (Calogero, Gallucci et al. 1988).

1.2.2 SSRIs remediate immunological and hematological changes in MDD

Studies have shown that both MDD and response to SSRIs can be mediated by hematological and immune cells and molecules, such as white blood cells and cytokines (Schiepers, Wichers et al. 2005). Treatment with SSRI remediates the increase or decrease in some white blood cell counts and cytokine levels in responders. For example, Kubera at al. found that SSRI (such as citalopram and sertraline), on one hand, inhibit the production of the pro-inflammatory cytokines, IL-1, IL-2, IL-6, tumor necrosis factor-alpha (TNF-α) and IFN-γ, that are increased in MDD patients (Kubera, Kenis et al. 2000). On the other hand, SSRI stimulate the production of IL-10, which is a negative immune-regulatory cytokine, which can normalize the levels of other cytokines (Kubera, Kenis et al. 2000). Likewise, many studies have shown that SSRIs, such as fluoxetine, restore T cell proliferation and the CD4/CD8 cell ratio to their normal levels in MDD patients (Frick, Rapanelli et al. 2009). Further, SSRIs decrease C-reactive protein (CRP), and leukocyte count to normal levels in MDD patients six weeks after treatment (Tuglu, Kara et al. 2003).

1.2.3 Classification of MDD patients according to the response to SSRIs

Many pharmacological antidepressants were developed to treat MDD. However, clinical trials have shown that only 30% of MDD patients respond to treatment with antidepressants (Howland 2008). Studies have shown that both MDD and response to SSRIs can be mediated by hematological and immune cells and molecules, such as white blood cells and cytokines (Schiepers, Wichers et al. 2005). However, treatment with SSRI does not normalize white blood cell counts and cytokine levels in non-responder patients with MDD. Previous studies have reported a clear implication of IL-2, IL-6 and IL-10 in MDD and response to SSRI. On the other hand, studies showed that non-depressed patients develop symptoms of depression when they receive IFN-γ treatment. Moreover, increased levels of some cytokines such as MIF were found in MDD patients without clearly knowing the rule of MIF in MDD. However, a little is known about the interaction of the immunological markers, cytokines, in MDD and/or response to SSRIs.

1.3 Brief Description of the Study

In this project, we aim to study white blood cell counts and certain cytokine levels in medication-naïve patients with MDD before and after treatment with SSRIs. In particular, we will investigate pro-inflammatory cytokines, including IL-2, IL-6, IFN-γ and macrophage MIF, and the anti-inflammatory cytokine IL-10. Normally, pro- and anti-inflammatory cytokines counteract for a balanced immune response (Figure1.1). Based on previous evidence, IL-1, IL-6 and IL-10 are involved in MDD and SRRI treatment. Moreover, treatment with cytokines like IFN-γ in non-depressed patients resulted in symptoms of depression. Likewise, elevated levels of MIF are associated with behavioral changes, including depressive symptoms. Nevertheless, the role of these cytokines and their interaction in MDD and response to SSRI is still not well clarified. We anticipate finding an imbalance among pro- and anti-inflammatory cytokines in MDD that might be normalized with SSRI treatment in responders, but not in non-responders (Figure1.2).Studying these specific markers that can *a priori* differentiate SSRI responders and non-responders will have a significant impact on our understanding of the pathophysiology of MDD and the mechanism of action of SSRI antidepressants (Edwards, Bosch et al. 2010, Bay-Richter, Janelidze et al. 2015). Further, this will provide the field of psychiatry with valuable clinical assessment tools that

can significantly advance patient-centric approaches in the treatment of MDD (Edwards, Bosch et al. 2010, Bay-Richter, Janelidze et al. 2015).

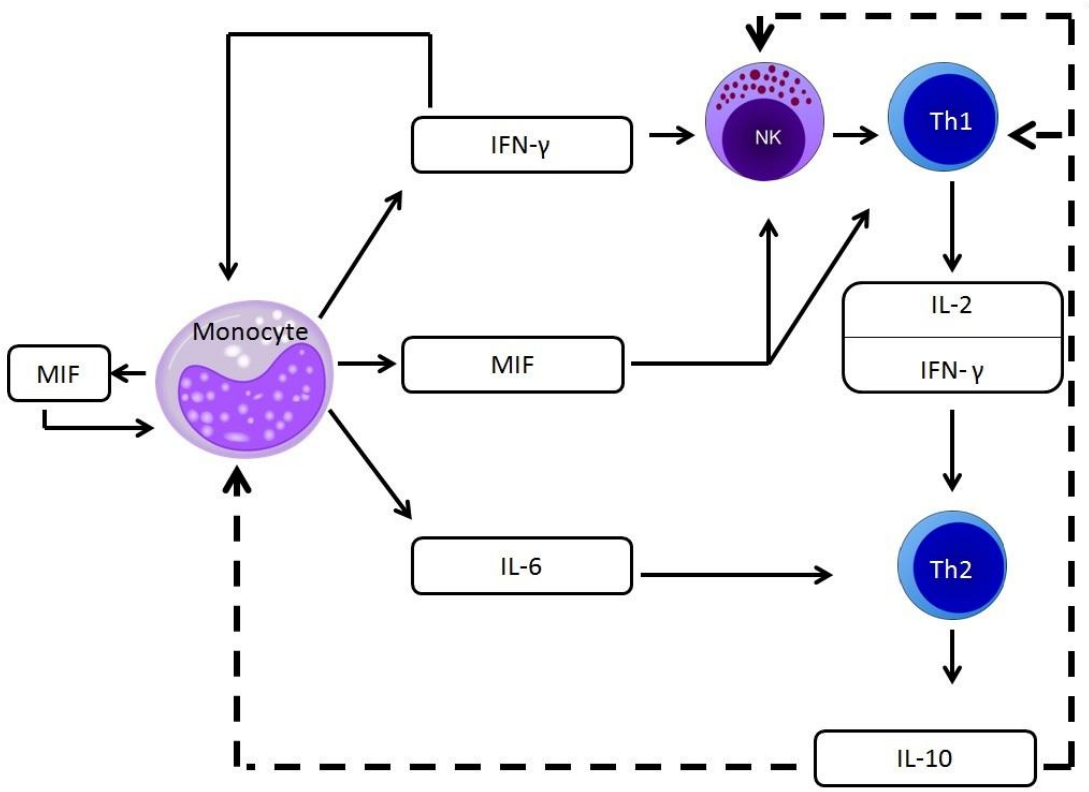


Figure1.1: The inflammatory response in the peripheral immune system. Interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-10 (IL-10), interferon gamma (IFN-γ), macrophage migration inhibitory factor (MIF), Th1: T-helper cell 1, Th2: T-helper cell 2, NK: natural killer cell, dashed line: inhibition, continuous line: stimulation.

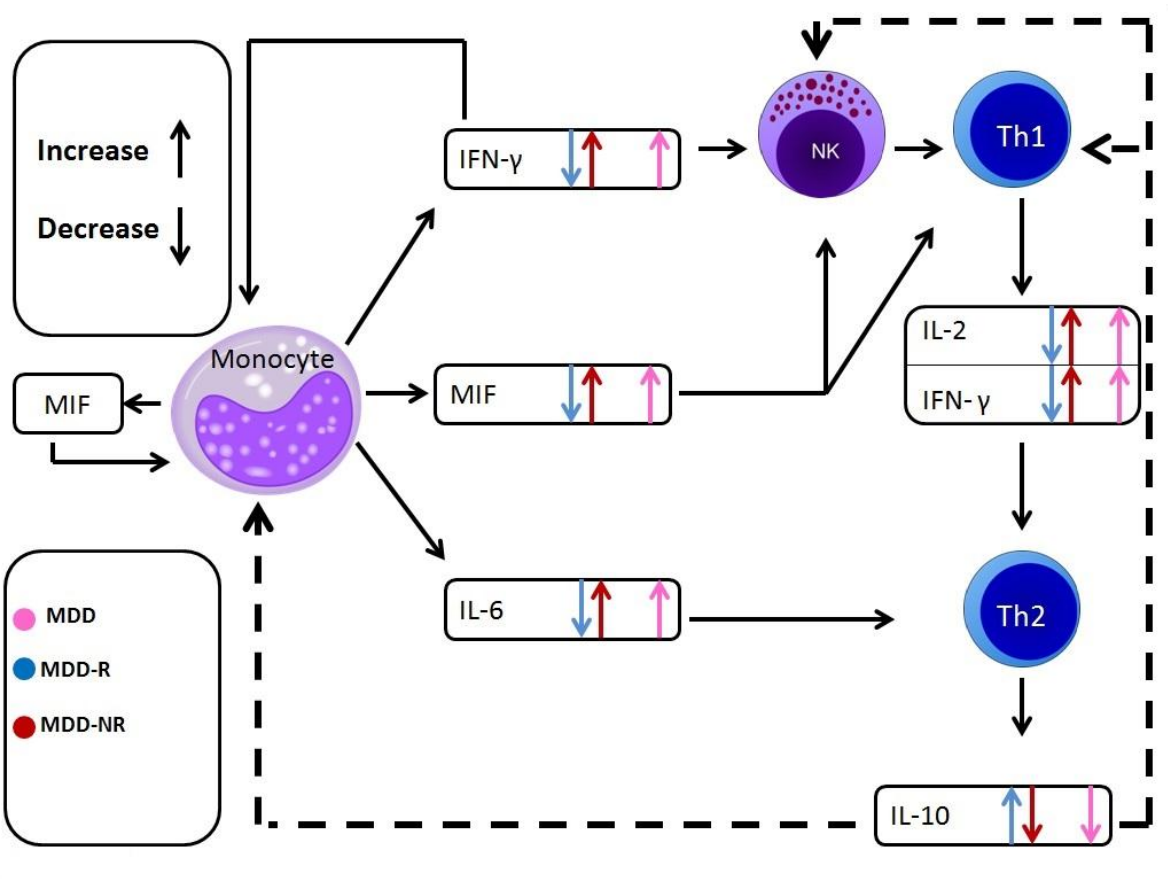


Figure1.2: The expected inflammatory response in the peripheral immune system in MDD and SSRI response. Interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-10 (IL-10), interferon gamma (IFN-γ), macrophage migration inhibitory factor (MIF), Th1: T-helper cell 1, Th2: T-helper cell 2, NK: natural killer cell, dashed line: inhibition, continuous line: stimulation.

1.4 Problem Statement

MDD is the most prevalent mental health problem in the West Bank, Palestine, affecting around 30% of Palestinians (Canetti, Galea et al. 2010, Madianos, Sarhan et al. 2012). Only 30% of MDD patients respond to SSRIs. Currently, psychiatrists have no valid tools to predict who will or will not respond to treatment among MDD patients.

1.5 Study Justification

Unfortunately, clinicians cannot predict who will or will not respond to SSRI antidepressants. If, however, a simple blood test in MDD patients could differentiate those who are, or are not,

likely to respond to subsequent SSRI administration, this would (1) provide immediate clinical relevance, helping to identify those most likely to benefit from SSRIs, and (2) inform future drug discovery by characterizing molecular and neural mechanisms associated with SSRI non-responders. This study will be the first to combine hematological (WBCs) and immunological (cytokines) markers in medication-naïve MDD patients to *a priori* predict response to SSRIs. This research can lead to clinically significant transformations of the field of psychiatry, which can ultimately improve treatment of MDD by translating emerging neuroscience knowledge into large-scale clinical trials and treatment protocols. Since only 30% of MDD patients receiving SSRIs show significant reduction in symptoms, it is of significant clinical importance to develop convenient diagnostic tools to *a priori* identify SSRI responders and non-responders.

1.6 Research Hypothesis

- IFN-γ and MIF, unlike IL-2, IL-6, and IL-10 could be potential immunological markers to *a priori* differentiate SSRI responders from non-responders in medication-naïve patients with MDD given their regulatory effects that could modulate the production of other cytokines.
- Counts of white blood cells that produce IFN-γ and MIF, namely monocytes and lymphocytes (T helper1) cells, could constitute hematological markers that *a priori* differentiate SSRI responders from non-responders in medication-naïve patients with MDD.

1.7 Study Questions

- Could IFN-γ and MIF *a priori* differentiate SSRI responders from non-responders in medication-naïve patients with MDD?
- Could monocyte and lymphocyte (T helper1) cell counts *a priori* differentiate SSRI responders from non-responders in medication-naïve patients with MDD?

1.8 Study Goal

- To identify hematological and immunological markers that can *a priori* predict response to SSRIs in medication-naïve patients with MDD.

1.9 Study Objectives

- To quantify IL-2, IL-6, IFN-γ, IL-10, and MIF in MDD patients at baseline (medication-naïve) and 4-6 weeks after receiving SSRIs.
    - These cytokines are the most widely implicated in MDD and response to SSRIs. Accordingly, we will use them as peripheral markers.
- To quantify CRP in MDD patients to control for the effect of inflammation.
- To determine the differential white blood cell counts (monocytes, lymphocytes, granulocytes; neutrophils, basophils, eosinophils) in patients with MDD before and after receiving SSRIs.

CHAPTER TWO

RESEARCH METHODOLOGY

2.1 Study Design and Framework

2.1.1 **Study Design:** Longitudinal study design.

2.1.2 **Sample Type:** Admission-based sample.

2.1.3 **Study Place:** West Bank, Palestine.

2.1.4 **Study Time:** August 2016 - October 2017.

2.2 Study Population

We tested patients with Major Depressive Disorder (MDD) recruited from psychiatric clinics in the West Bank, Palestine.

2.3 Ethical Considerations

This research was conducted according to the Declaration of Helsinki and approved by the Al-Quds Ethics Committee. Written informed consent was obtained from all subjects before protocol-specified procedures were carried out. The approval letter is available upon request.

2.4 Assessment Criteria Including Materials and Methods for Each Variable

In this longitudinal study, we recruited 8 medication-naïve patients with MDD from different psychiatric clinics in the West Bank, Palestine. These patients never received any form of treatment. Further, 8 matched healthy control subjects were recruited for this study.

Patients with MDD were tested twice; (1) At baseline session, upon diagnosis with MDD, and (2) 4-6 weeks after receiving SSRI treatment (estimated time for clinical improvement). Healthy subjects were also tested twice 4-6 weeks apart. A peripheral blood sample was collected using an EDTA tube (10 mL) from each subject per session. Each blood sample was used for several tests as follows: (1) blood film preparation: 1mL of the blood sample, within two hours, and (2) plasma: the rest of the EDTA blood sample was spun at 1000g for 10

minutes then the plasma was harvested and stored at -70°C for cytokine and quantitative CRP tests.

2.5 Sample Testing Methods and Devices

In this study, we are interested in the following cytokines: IL-2, IL-6, IL-10, IFN-γ, MIF as well as CRP quantification. To measure the levels of these analytes, we used enzyme-linked immune-sorbent assay (ELISA) kits. This is the most commonly used assay to quantify CRP and cytokine levels as reported in several studies of MDD (Maes, Meltzer et al. 1995) and SSRI antidepressants (Hernandez, Mendieta et al. 2008) on cytokine levels. To quantify cytokine results we used a semi-automated washer system device. For CRP results we used an automated ELISA device (NycoCard READER II).

We prepared the blood films manually using appropriate glass slides. Then, we stained them using a rapid stain "RAPIHEM". We examined the stained blood films using light microscopy in order to obtain the differential white blood cell count. Table2.1 below summarizes the materials and methods that we used.

Table 2.1: The Used Materials and Methods.

Test Name	Materials	Material manufacture	Method	Device
Human IL-2 Cytokine	Human IL-2 Mini TMB ELISA Development KIT	PEPROTECH	ELISA	Semi-automated washer system
Human IL-6 Cytokine	Human IL-6 Mini TMB ELISA Development KIT	PEPROTECH	ELISA	Semi-automated washer system
Human IFN-γ Cytokine	Human IFN-γ Mini ABTS ELISA Development KIT	PEPROTECH	ELISA	Semi-automated washer system
Human IL-10 Cytokine	Human IL-10 Mini ABTS ELISA Development KIT	PEPROTECH	ELISA	Semi-automated washer system
Human MIF cytokine	MIF (Human ELISA Kit)	BioVison	ELISA	Semi-automated washer system
Quntitative CRP	NycoCard CRP	Alere	ELISA	Aautomated ELISA device (NycoCard READER II)
Blood Film	- Glass Slides - Rapid stain "RAPIHEM"	-	Manually	Light Microscopy
Complementary materials	- ELISA Buffer Kit (TMB) - ELISA Buffer Kit (ABTS) - Multi-channel pipette(8 channels, 30-300 µl)	- PEPROTECH - PEPROTECH - ThermoFisher Scientific	-	-

2.6 Sample Processing

The frozen plasma samples at -70°C were thawed at room temperature using tap water.

2.7 Test Protocols

2.7.1 Human cytokine ELISA kits Protocol

All samples were tested using ELISA kits for MIF, IFN-γ, IL-2, IL-6, and IL-10 cytokines according to the manufacturer instructions.

2.7.2 Quantitative CRP Test Protocol

In the first step, 5 µl of subject plasma were diluted in the provided diluent (borate buffer and detergents) in a specific tube by mixing them for 10 seconds. Then, 50 µl of the diluted sample were applied to the test device (a plastic device containing a membrane coated with monoclonal anti-CRP antibodies). The sample was allowed to soak into the membrane for approximately 30 seconds. One drop of the conjugate (monoclonal anti-CRP antibodies labeled with ultra-small gold particles) was added to the test device and was allowed to be soaked for approximately 30 seconds. After that, one drop of the washing solution (phosphate buffered NaCl solution and detergents) was added to the test device and was allowed to soak for 20 seconds. Finally, the results were read using the NycoCard READER II.

2.7.3 Blood Film Preparation Protocol

On a slide, 5 µl of EDTA blood sample was applied and appropriately spread. Then, the film was left to dry. The slide was stained using Rapid stain "RAPIHEM". The prepared blood film was read a differential count of the white blood cells (eosinophils, basophils, neutrophils, monocytes, and lymphocytes) using a light microscopy.

2.8 Data Analysis

For analysis of the levels of cytokine and white blood cell data, we used mixed-model MANOVA where testing session (baseline, retest) is a within-subject variable, group (responder, non-responder, control) is a between-subject variable, and cytokine levels and white blood counts are the dependent variables. Further, we used the Kolomogorov-Smirnov test to examine normality of the dependent variables at both testing sessions. We used IBM© SPSS 20.0 to analyze the data.

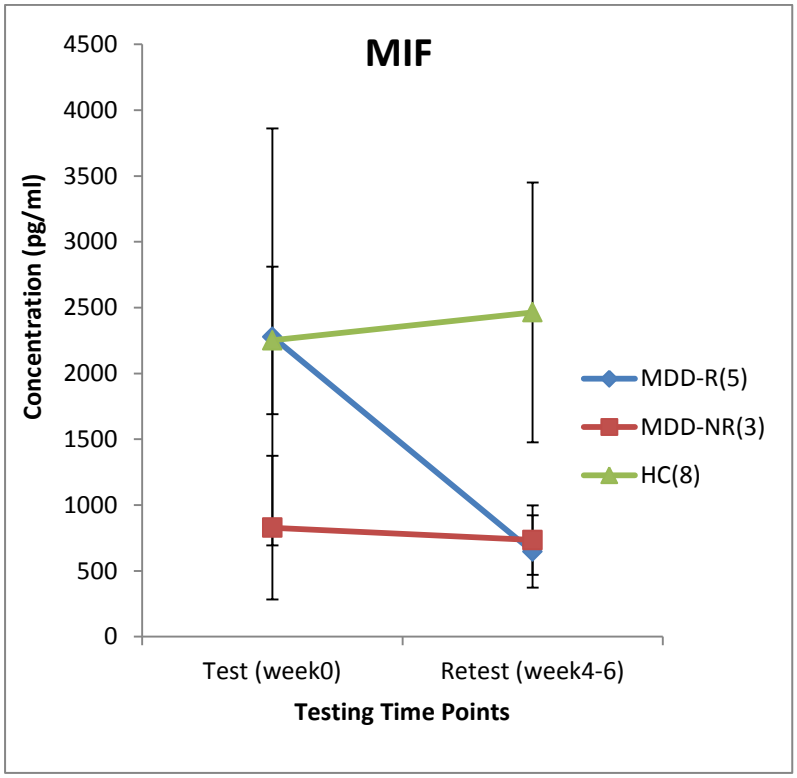
CHAPTER THREE

STUDY RESULTS

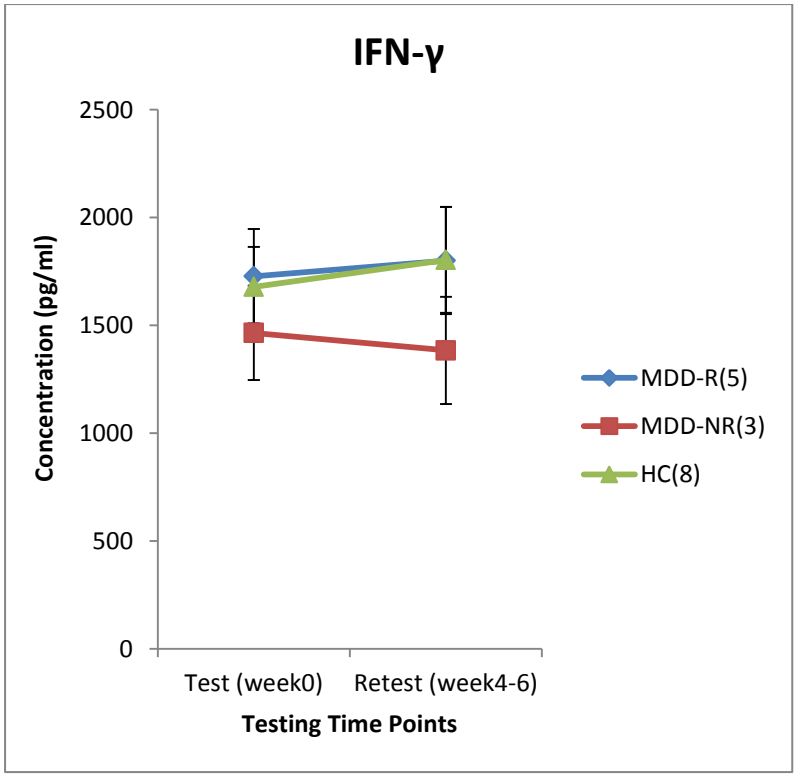
For each subject at each testing session, we obtained 10 results; 5 immunological markers, and 5 hematological markers. We used the Kolomogorov-Smirnov test to examine normality of the dependent variables at both testing sessions. Almost all variables were normally distributed except for eosinophil and basophil counts. Hence, we decided to use parametric statistical tests to examine our data.

We used a mixed-model MANOVA to analyze our data, with group (MDD responder, MDD non-responder, and HC) as the between-subject variable, blood markers and testing sessions as the within-subject variables, and immunological (MIF, IFN-γ, IL-2, IL-6, and IL-10) and hematological (eosinophils, basophils, neutrophils, monocytes, and lymphocytes) marker concentrations as dependent variables. Multivariate tests revealed a significant effect of blood marker. Univariate within-subject comparisons showed a significant effect of blood markers ($F(8,104)=12.857$, $p<0.001$, $\eta^2=0.497$), a significant interaction between group and blood marker ($F(8,104)=1.758$, $p=0.047$, $\eta^2=0.201$). Figure 3.1 (A-E) show concentrations of specific cytokines (MIF, IFN- γ, IL-2, IL-6, and IL-10 respectively) in the three tested groups at session 1 (week-0) and session 2 (week 4-6). Figure 3.2 (A-E) show absolute count of specific hematological markers (eosinophils, basophils, neutrophils, monocytes, and lymphocytes respectively) in the three tested groups at session 1 (week-0) and session 2 (week 4-6).

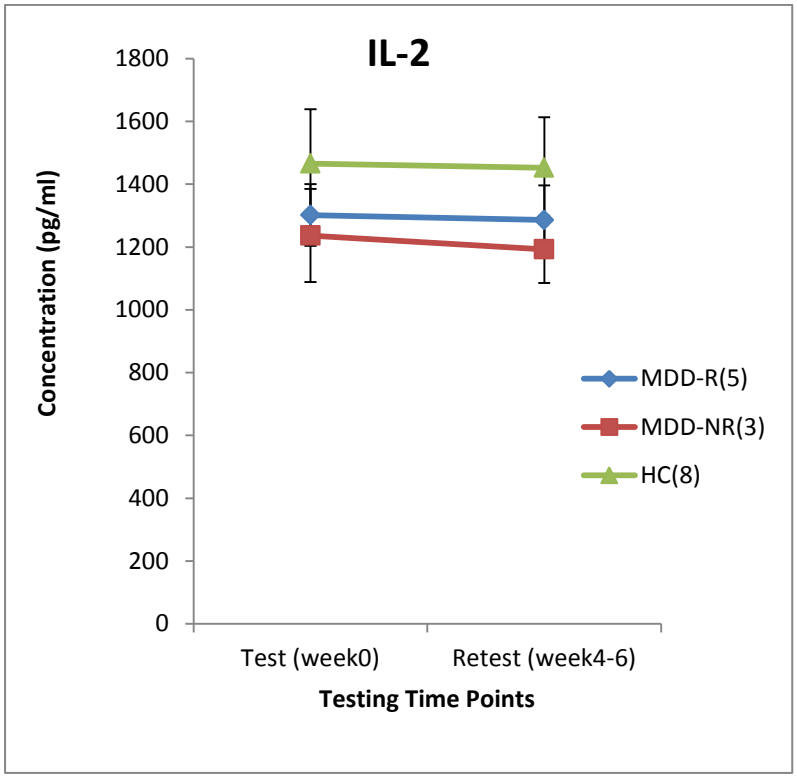
A


MIF
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Concentration (pg/ml)
Test (week0)
Retest (week4-6)
Testing Time Points
MDD-R(5)
MDD-NR(3)
HC(8)

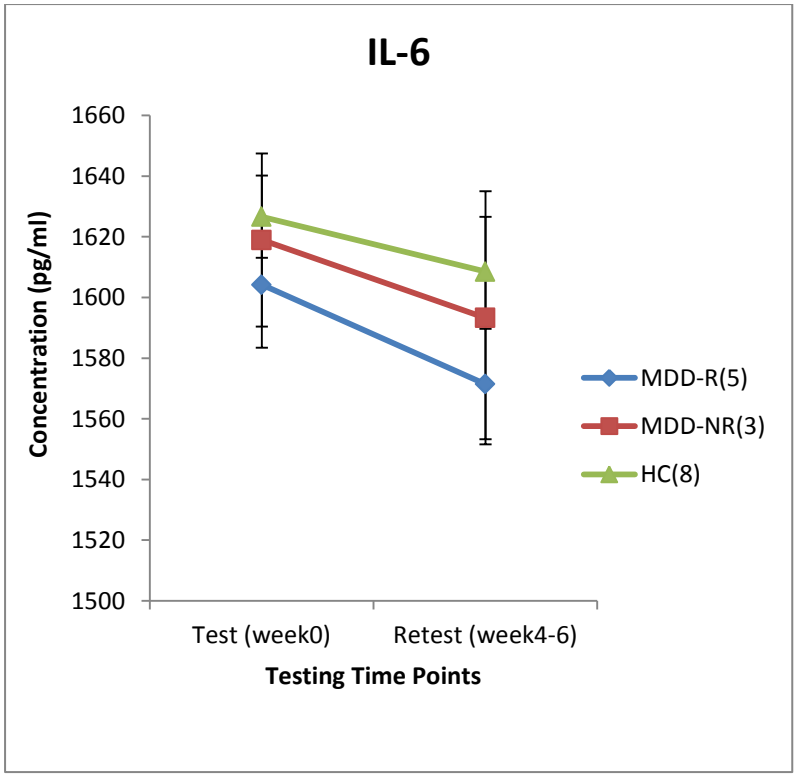
B


IFN-γ
2500
2000
1500
1000
500
0
Concentration (pg/ml)
Test (week0)
Retest (week4-6)
Testing Time Points
MDD-R(5)
MDD-NR(3)
HC(8)

C


IL-2
1800
1600
1400
1200
1000
800
600
400
200
0
Concentration (pg/ml)
Test (week0)
Retest (week4-6)
Testing Time Points
MDD-R(5)
MDD-NR(3)
HC(8)

D


IL-6
1660
1640
1620
1600
1580
1560
1540
1520
1500
Concentration (pg/ml)
Test (week0)
Retest (week4-6)
Testing Time Points
MDD-R(5)
MDD-NR(3)
HC(8)

E

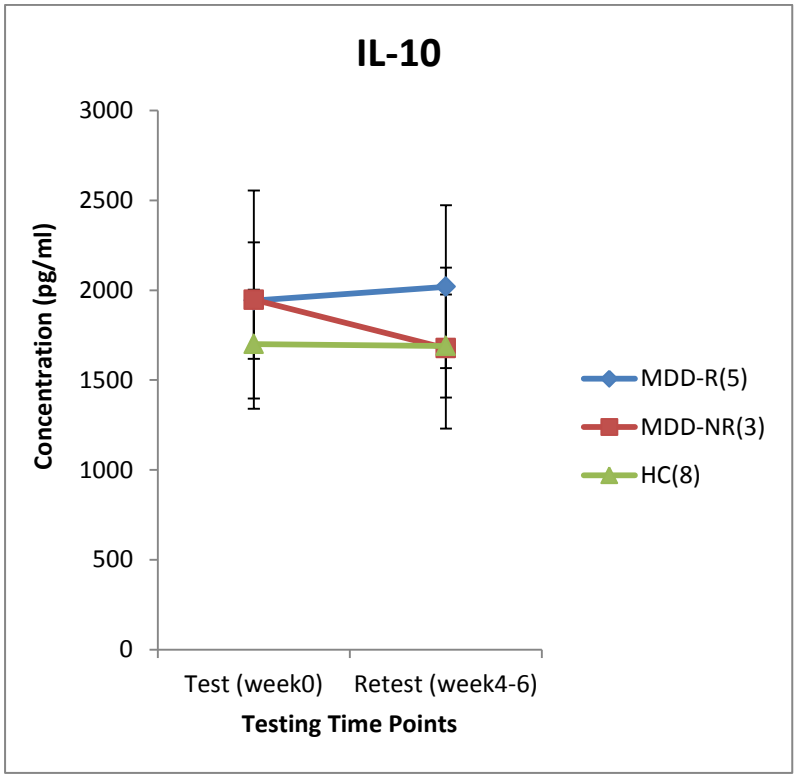
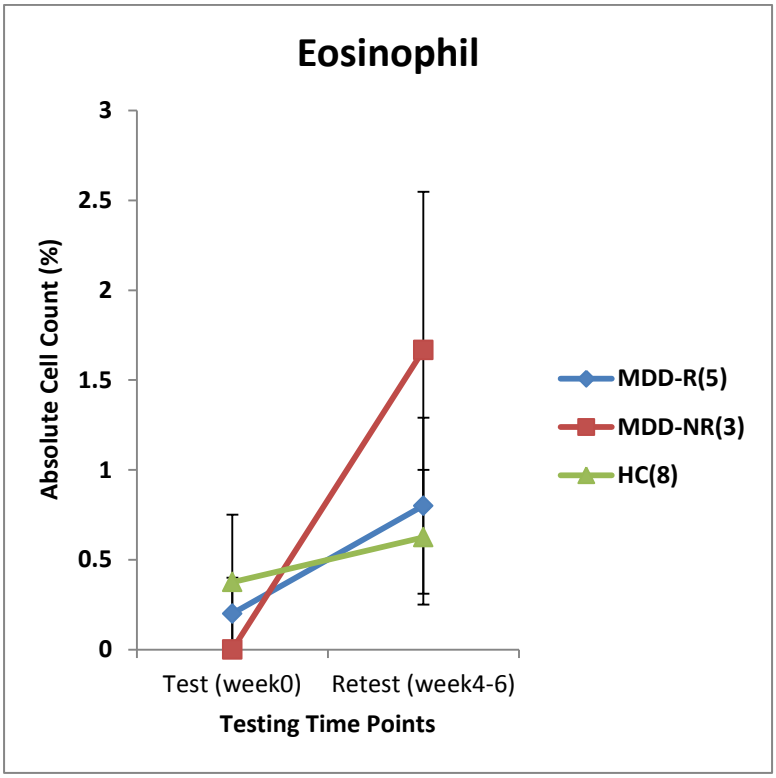
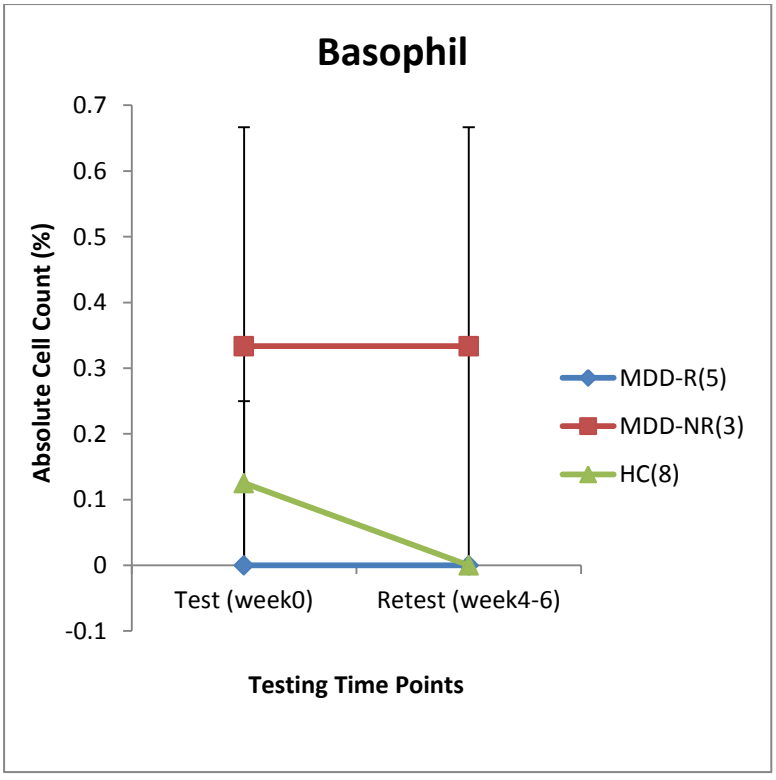


Figure3.1 (A-E): The concentrations of specific cytokines (MIF, IFN- γ, IL-2, IL-6, and IL-10, respectively) in the three tested groups at session 1 (week-0) and session 2 (week 4-6).

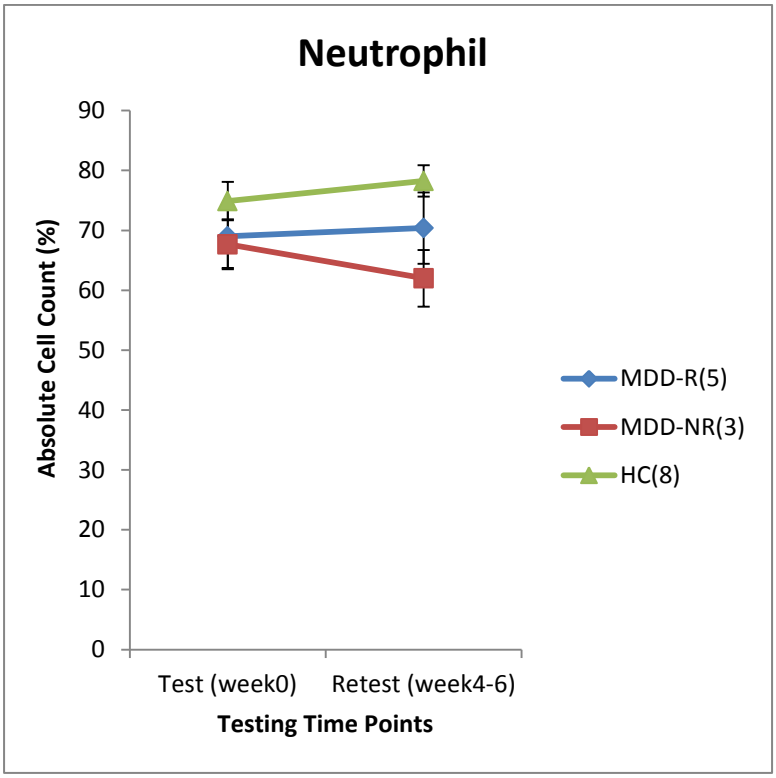
A


Eosinophil
3
2.5
2
1.5
1
0.5
0
Absolute Cell Count (%)
Test (week0)
Retest (week4-6)
Testing Time Points
MDD-R(5)
MDD-NR(3)
HC(8)

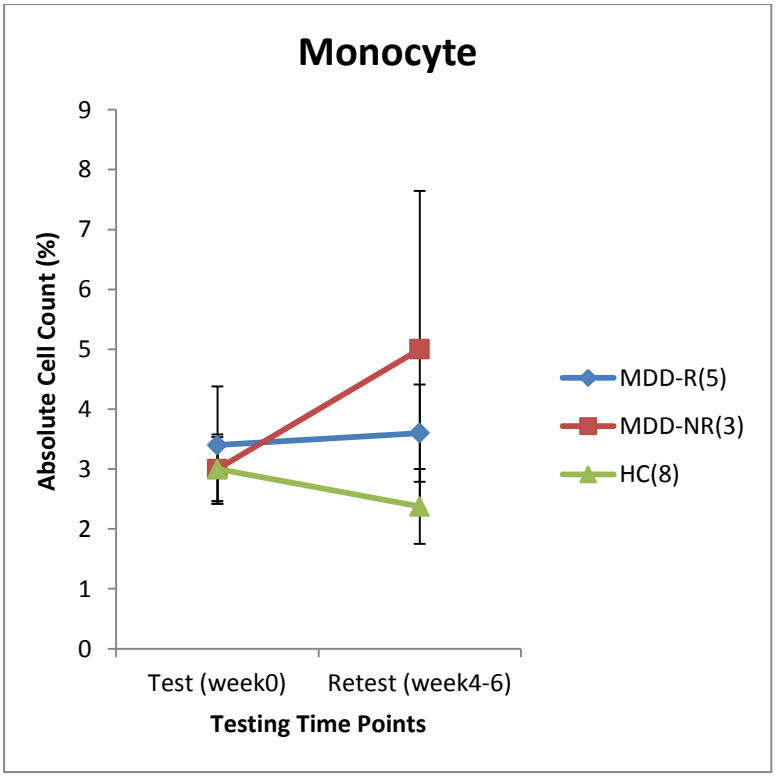
B


Basophil
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
Absolute Cell Count (%)
Test (week0)
Retest (week4-6)
Testing Time Points
MDD-R(5)
MDD-NR(3)
HC(8)

C


Neutrophil
90
80
70
60
50
40
30
20
10
0
Absolute Cell Count (%)
Test (week0)
Retest (week4-6)
Testing Time Points
MDD-R(5)
MDD-NR(3)
HC(8)

D


Monocyte
9
8
7
6
5
4
3
2
1
0
Absolute Cell Count (%)
Test (week0)
Retest (week4-6)
Testing Time Points
MDD-R(5)
MDD-NR(3)
HC(8)

E

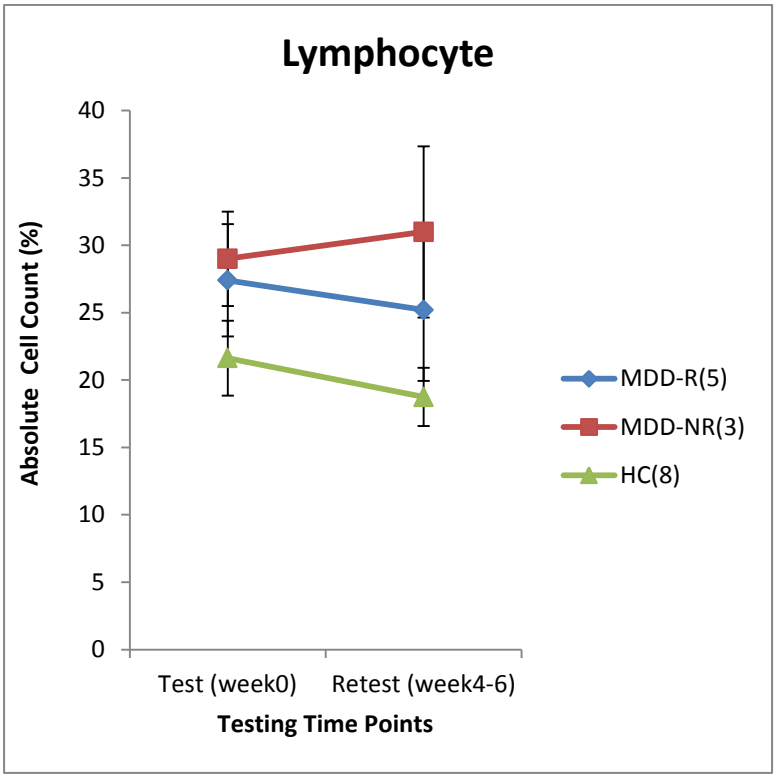
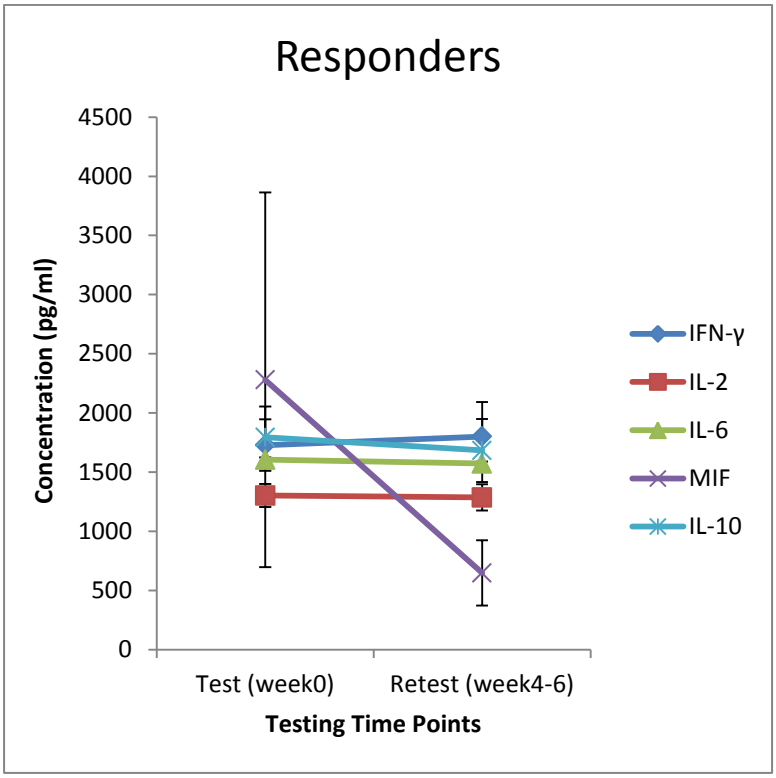


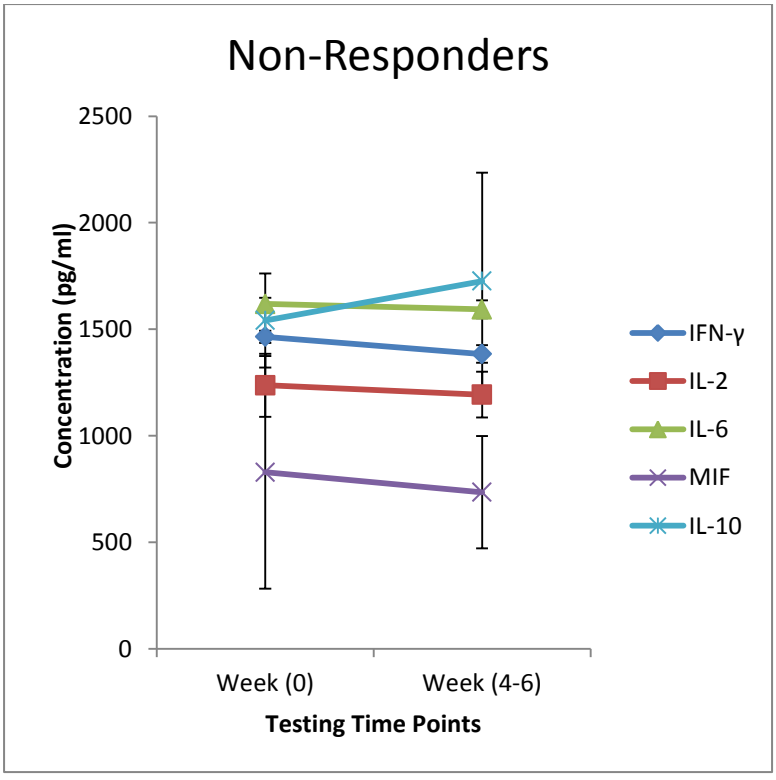
Figure3.2 (A-E): The absolute count of specific hematological markers (eosinophils, basophils, neutrophils, monocytes, and lymphocytes, respectively) in the three tested groups at session 1 (week-0) and session 2 (week 4-6).

To investigate the interaction between group and blood marker, we used two mixed-model ANOVAs with group as the between-subject variable, testing session and type of blood markers as the within-subject variables, and blood marker concentrations as the dependent variables. To protect the level of significance, we used a Bonferroni corrected experiment-wise $\alpha=0.025$. ANOVAs did not show any significant effects, mainly due to the low number of subjects included in the models. Figure 3.3(A-C) illustrates concentrations of all cytokines in the three groups: SSRI responders, SSRI non-responders and healthy controls respectively. Figure3.4 (A-C) illustrates absolute count of all hematological markers in the three groups: SSRI responders, SSRI non-responders and healthy controls respectively.

A


Responders
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Concentration (pg/ml)
Test (week0)
Retest (week4-6)
Testing Time Points
IFN-γ
IL-2
IL-6
MIF
IL-10

B


Non-Responders
2500
2000
1500
1000
500
0
Concentration (pg/ml)
Week (0)
Week (4-6)
Testing Time Points
IFN-γ
IL-2
IL-6
MIF
IL-10

C

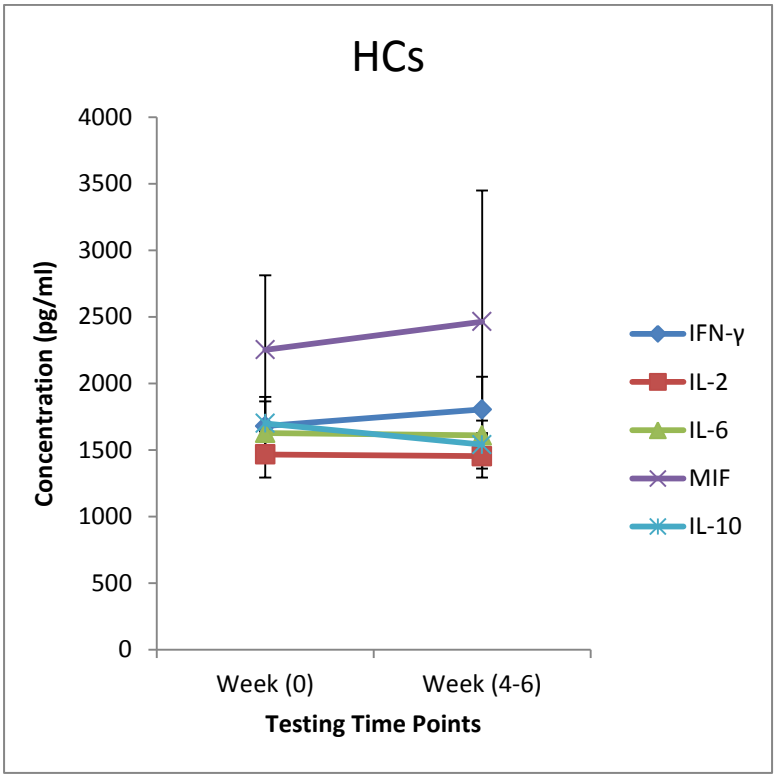
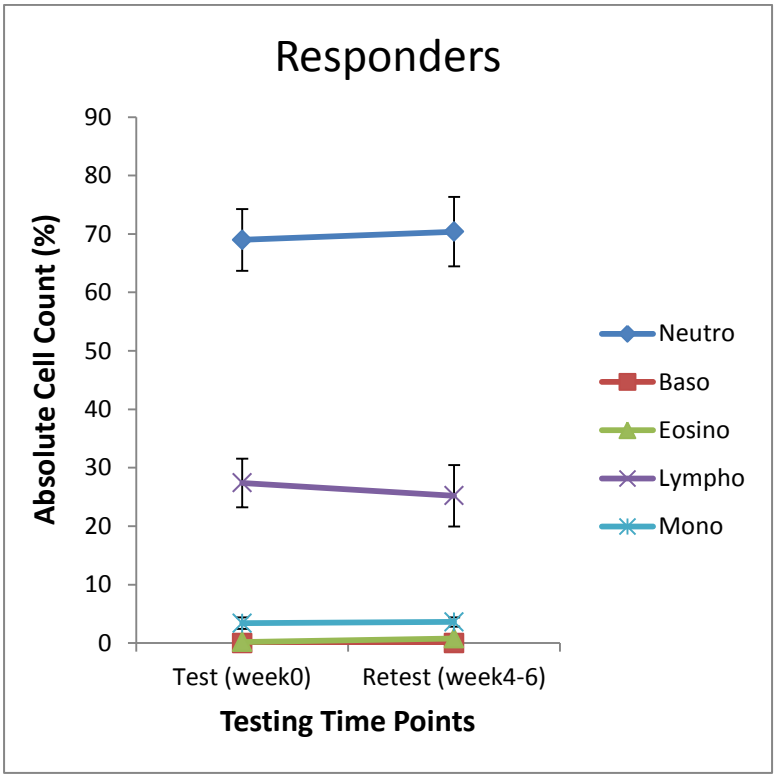
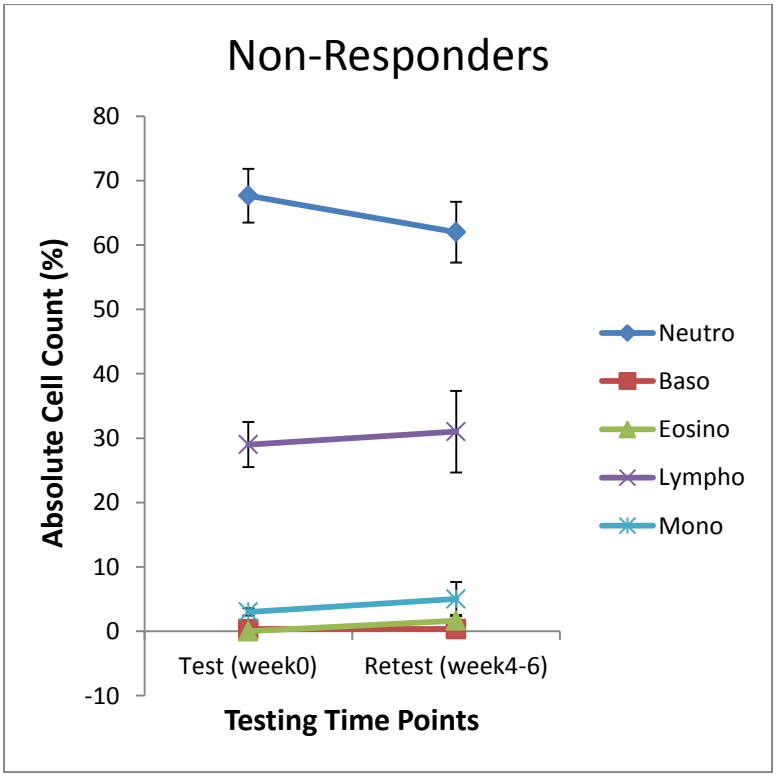


Figure 3.3(A-C): The concentrations of all cytokines in the three groups: SSRI responders (A), SSRI non-responders (B) and healthy controls (C).

A


Responders
Absolute Cell Count (%)
90
80
70
60
50
40
30
20
10
0
Test (week0)
Retest (week4-6)
Testing Time Points
Neutro
Baso
Eosino
Lympho
Mono

B


Non-Responders
Absolute Cell Count (%)
80
70
60
50
40
30
20
10
0
-10
Test (week0)
Retest (week4-6)
Testing Time Points
Neutro
Baso
Eosino
Lympho
Mono

C

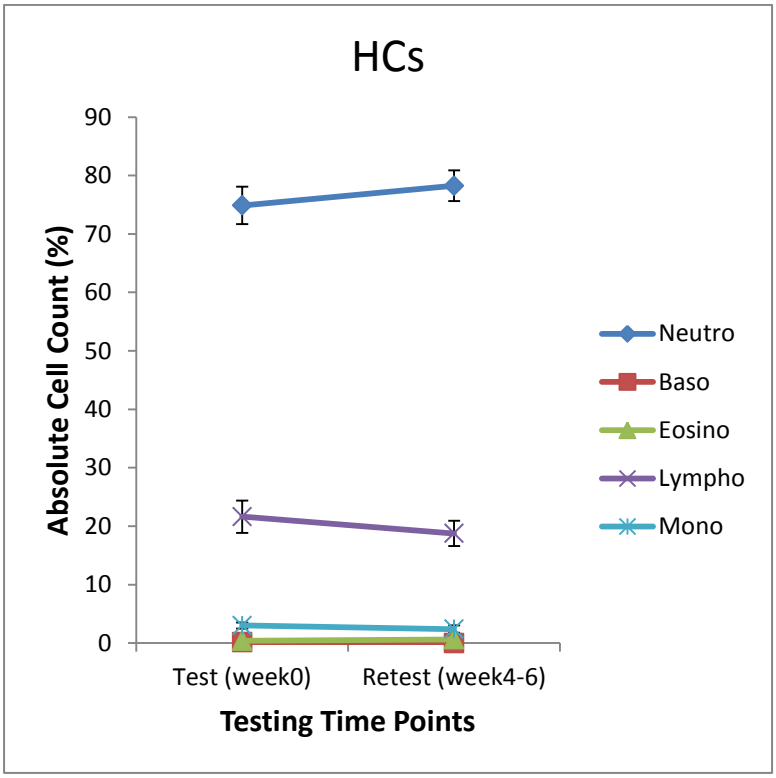


Figure 3.4(A-C): The absolute count of all hematological markers in the three groups: SSRI responders (A), SSRI non-responders (B) and healthy controls (C).

CHAPTER FOUR

DISCUSSION

In this thesis, we investigated different pro- and anti-inflammatory cytokines as well as hematological markers in two groups: (1) patients with MDD and (2) healthy controls. Patients were tested as medication-naïve at baseline and after receiving SSRI antidepressants for 4-6 weeks. Healthy subjects were tested and retested on a similar timeline. Our results show that there is a trending increase in pro-inflammatory cytokine levels and a decrease in the anti-inflammatory cytokine levels in medication-naïve patients with MDD. However, the degree of increase or decrease varied between SSRI responders and non-responder both at baseline and after treatment with SSRI. Further, we found that the WBC differential counts differed between MDD responders and non-responders vs. healthy controls both at baseline and after treatment with SSRI. The concentrations of the quantitative CRP were in the reference range for all tested MDD patients and healthy controls, which might indicate that all cytokine level and cell count variations, did not result from a generalized inflammatory response. However, we acknowledge that this is a preliminary study with a small number of subjects. Therefore, it is currently difficult to draw clear conclusions about changes in cytokines and hematological markers in MDD and in response to treatment.

4.1 Medication-Naïve MDD Patients

Evidence suggests that MDD exhibited imbalanced interaction between the pro-inflammatory and the anti-inflammatory cytokines that may result from interruptions in the immunological pathways (Myint, Leonard et al. 2005, Lee and Kim 2006) (Lee & Kim, 2006). At baseline, all MDD patients (future responders and non-responders) showed high concentrations of the pro-inflammatory cytokines in line with previous studies in the literature (Maes, Meltzer et al. 1995, Capuron, Gumnick et al. 2002, Leyton-Jaimes, Kahn et al. 2017). Further, our findings indicate that, at baseline, cytokine concentrations in SSRI non-responders were lower than those of healthy controls and SSRI responders. In particular, this was clear in the concentrations of the pro-inflammatory cytokines IFN-γ, MIF, and IL-2. However, the

concentrations of IL-6 were lower in SSRI responders than those in SSRI non-responders at baseline (before treatment). Moreover, the concentrations of the anti-inflammatory cytokine IL-10 were lower in SSRI non-responders than SSRI responders and healthy controls. This corresponds with our hypothesis that anti-inflammatory cytokines, such as IL-10, could show *a priori* differences between responders and non-responder, given their role in normal immune response (Schiepers, Wichers et al. 2005).

Previous research found that the immune activation in MDD includes increasing number of white blood cells (Maes, Meltzer et al. 1995). Moreover, some studies showed that the white blood cell counts can change in the response to antidepressants in MDD patients (Schiepers, Wichers et al. 2005). In our results, replicating previous literature, the absolute lymphocyte count was higher in medication-naïve MDD patients than in healthy controls as expected (Maes, Meltzer et al. 1995). Overall, our results show that the level of cytokines in MDD patients at baseline may be different in SSRI responders compared to SSRI non-responders before starting treatment. There are no studies in the literature that differentiate MDD patients who are SSRI responders from those who are SSRI non-responders in the medication-naïve state. Figure 4.1 summarizes the cytokine levels in MDD patients at baseline session.

4.2 On SSRI MDD Patients

Normally, the pro-inflammatory cytokines interact with the anti-inflammatory cytokines to maintain a balance in the immune response. In other words, the production of the pro-inflammatory cytokines by macrophages or monocytes is involved directly or indirectly in the inflammatory process. The anti-inflammatory cytokines are produced to suppress the immune response by counteracting cellular activation as well as the production of pro-inflammatory cytokines to maintain the immune response (Schiepers, Wichers et al. 2005). This is what is expected to occur in SSRI responders after receiving SSRI: an increase in the levels of anti-inflammatory cytokines to counter act the increased levels of pro-inflammatory cytokines in MDD patients.

In this study, after 4-6 weeks of treatment with SSRI, IFN-γ and IL-2 levels did not change in SSRI responders versus SSRI non-responders. However, MIF levels were decreased in SSRI responders but not in SSRI non-responders. This finding is in line with those of Cattaneo et al.

(Cattaneo, Gennarelli et al. (2013)); however, our results are shown in medication naïve MDD patients while Cattaneo et al. did their study in off-medication MDD patients. IL-6 levels were decreased in both patient groups. However, IL-6 concentrations in SSRI responders were even lower than those in SSRI non-responders. The concentrations of the anti-inflammatory cytokine IL-10 were decreased in SSRI non-responders but did not change in SSRI responders. Kubera at al. showed that SSRI stimulate the production of IL-10, which can normalize the levels of other cytokines. They also found that SSRI prevent the production of the pro-inflammatory cytokines, IL-1, IL-2, IL-6, tumor necrosis factor-alpha and IFN-γ (Kubera, Kenis et al. 2000). In our results, however, we only observed a decrease in pro-inflammatory cytokines MIF and IL-6 levels following SSRI administration. It might be the case that normalization of pro-inflammatory cytokines takes longer than normalization of clinical symptoms. Further, the limited number of subjects per group in our study limits our ability to reach concrete conclusions. Figure 4.2 summarizes cytokine level variations in MDD patients after 4-6 weeks of receiving SSRI.

The lymphocyte count decreased only in SSRI responders 4-6 weeks after administration of SSRI. Frick et al. showed that SSRI restore T cell proliferation and the CD4/CD8 cell ratio to their normal levels in MDD patients (Frick, Rapanelli et al. 2009). However, SSRI non-responders showed increased number of lymphocytes following SSRI administration. The monocyte counts increased in SSRI non-responders but they did not change in SSRI responders. The increase and/or decrease in the cell counts might be related to cytokine level modulation in MDD and in response to SSRI since lymphocytes and monocytes are implicated in the cytokines production. Figure 4.3 shows the variations that occurred in cell counts before and after SSRI treatment in MDD patients.

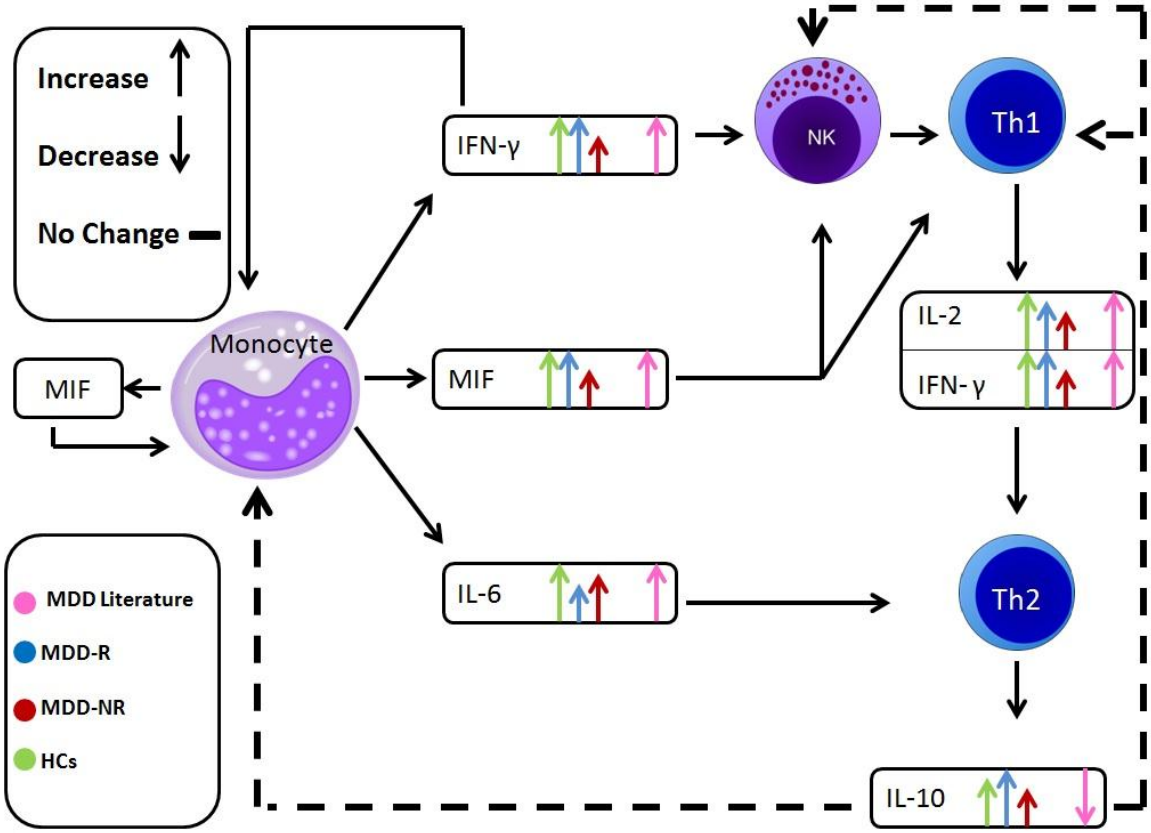


Figure4.1: Cytokines levels in MDD patients at baseline. Interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-10 (IL-10), interferon gamma (IFN-γ), macrophage migration inhibitory factor (MIF), Th1: T-helper cell 1, Th2: T-helper cell 2, NK: natural killer cell, dashed line: inhibition, continuous line: stimulation.

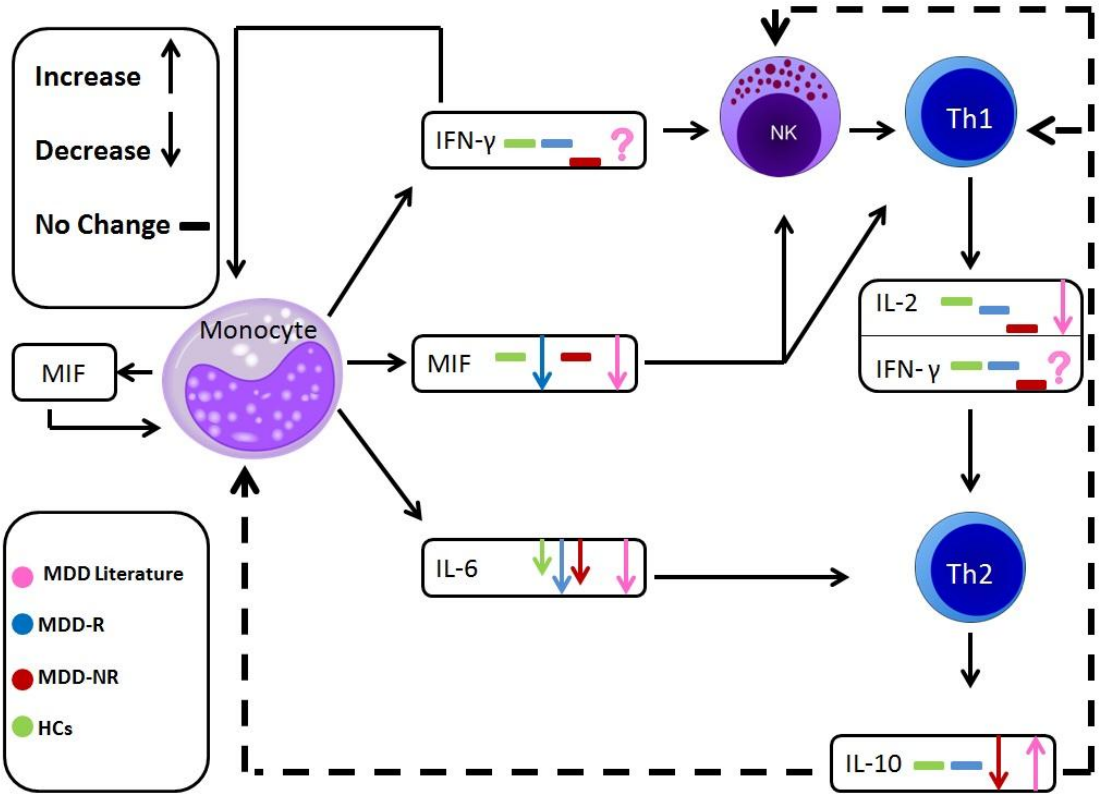


Figure4.2: Cytokines levels in MDD patients after 4-6 weeks of SSRI treatment. Interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-10 (IL-10), interferon gamma (IFN-γ), macrophage migration inhibitory factor (MIF), Th1: T-helper cell 1, Th2: T-helper cell 2, NK: natural killer cell, dashed line: inhibition, continuous line: stimulation.

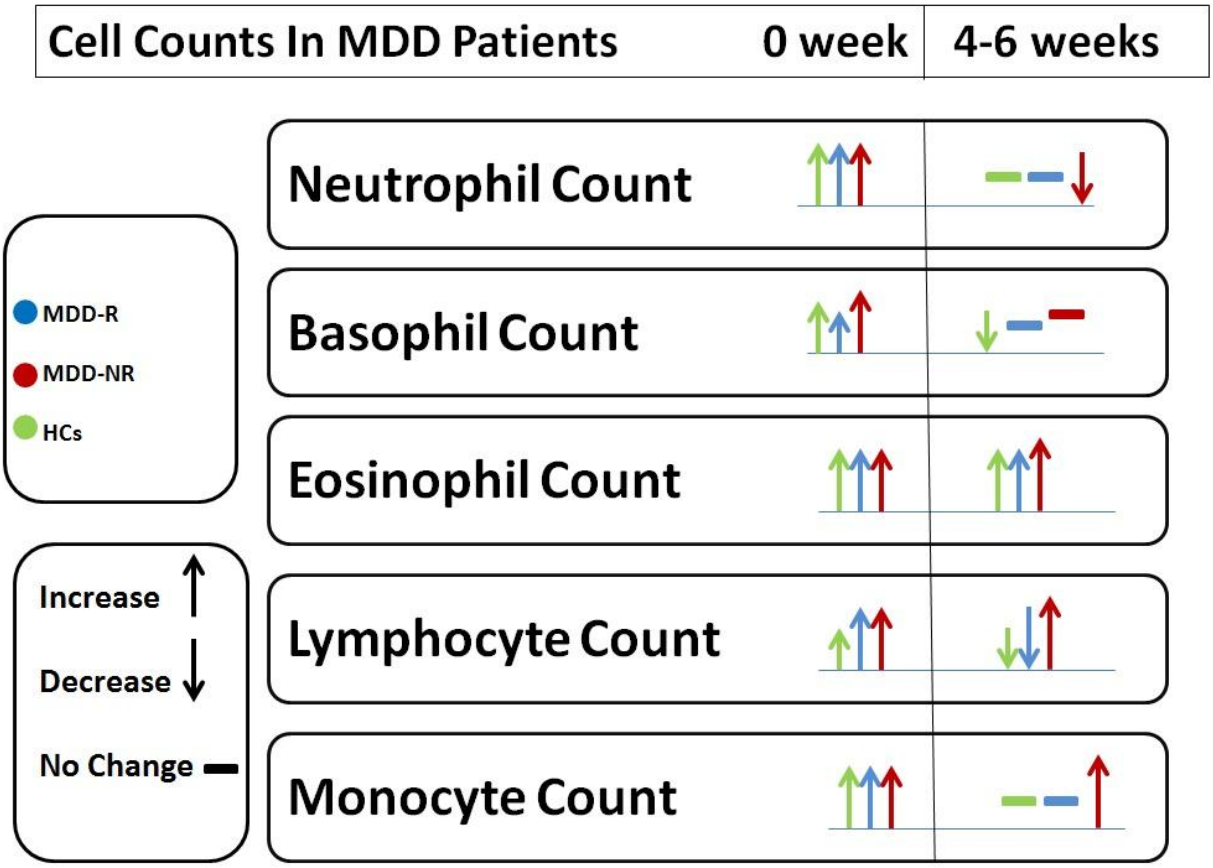


Figure4.3: Cell counts in MDD patients at baseline and after 4-6 weeks of SSRI treatment. Interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-10 (IL-10), interferon gamma (IFN-γ), macrophage migration inhibitory factor (MIF), Th1: T-helper cell 1, Th2: T-helper cell 2, NK: natural killer cell, dashed line: inhibition, continuous line: stimulation.

4.3 Limitations

The major limitation in our study is the low number of recruited subjects. This limited our ability to make clear conclusions about the current findings. We anticipate that with a larger sample we will have sufficient statistical power to find significant differences and be able to use statistical classification models to *a* priori differentiate SSRI responders and non-responders. Further, we only investigated a sample of cytokines implicated in MDD. There are many other cytokines implicated in MDD but were not included. Moreover, we tested MDD

patients after careful recruitment and diagnosis. However, structural and functional changes that are associated with comorbidities in MDD patients (e.g. comorbid anxiety in 80% of patients) might also play a role in shaping our results. Also, we examined MDD as a uniform entity; however, many studies consider MDD to be a spectrum that encompasses a group of different disorders based on symptom clusters. Finally, we had limited access to molecular and neural data that could explain the potential difference between responders/non-responders.

4.4 Conclusion and Future Directions

In summary, the results are indicative that there is an implication of cytokines as well as WBC counts in MDD and in response to SSRI with a clear interaction between the pro- and the anti-inflammatory components of the immune response. Future efforts will focus on increasing the sample are needed to support these results and to clarify the pathophysiology of the immune activity in MDD and the response to SSRI. A future study can investigate the effects of chronic treatment with SSRIs and other antidepressants on MDD patients as well as on cytokine levels. Further, we aim to study the effect of antidepressants at different time points, not only one, after treatment initiation in MDD patients.

دراسة استجابة مرضى الاكتئاب السريري لمثبطات استرجاع السيروتونين الانتقائية من خلال جزيئات مناعية و دموية معينة

إعداد: رحمة يوسف سعيد النتشة.

المشرف الأول: د محمد مصطفى حرزالله.

المشرف المشارك: د محمود عبد الرحمن سرور.

الملخص

يُعرّف الاكتئاب السريري على أنه اضطراب نفسي يتصف بطول فترة الحزن، والمزاج المحبط، بالإضافة إلى فقد اللذة والسعادة في شتى مناحي الحياة. وهو أكثر المشاكل الصحية النفسية انتشاراً في الضفة الغربية -فلسطين، حيث يؤثر على ما يقارب 30% من الشعب الفلسطيني. تعتبر مثبطات استرجاع السيروتونين الانتقائية أكثر مضادات الاكتئاب المستخدمة لعلاج مرضى الاكتئاب السريري، علماً بأنّ 30% فقط من مرضى الاكتئاب يستجيبون لهذه المضادات. حتى الآن، ليس هناك أي أدوات أو فحوصات طبية قادرة على الكشف عن إمكانية استجابة مريض الاكتئاب من عدم استجابته لمثبطات استرجاع السيروتونين الانتقائية قبل البدء في العلاج. في هذا السياق، طرحت العديد من الدراسات السابقة إمكانية حدوث الاكتئاب السريري نتيجة لخلل ما في مسارات مناعية وخلوية داخل جسم المريض. لذا، في هذه الدراسة نهدف إلى دراسة جزيئات مناعية وخلوية معينة قد تمكننا من معرفة المستجيب من غير المستجيب لمثبطات استرجاع السيرتونين الانتقائية قبل أن يبدأ المريض العلاج، مما يسهل على الطبيب المختص اختيار العلاج المناسب للمريض المناسب.

شارك في هذه الدراسة ثمانية من مرضى اكتئاب سريري من عيادات نفسية مختلفة في الضفة الغربية ولم يكن أيٌّ من المرضى المشاركين قد عولج من قبل لأجل الاكتئاب السريري. كما شارك في هذه الدراسة نفس العدد من الأشخاص الأصحاء السليمين من الاكتئاب السريري. جُمعت عينات دم (10 مل) من مرضى الاكتئاب المشاركين عند تشخيص المرض و مرة أخرى بعد تلقيهم مثبطات استرجاع السيروتونين الانتقائية بمدة أربعة إلى ستة أسابيع. وقد تمّ تقسيم المرضى إلى مستجيبين للعلاج وغير

مستجيبين حسب تقييمهم بعد فترة العلاج. أما المشاركون الأصحّاء فقد تم أخذ عيناتهم في المرة الأولى ثم بعدها بأربعة إلى ستة أسابيع. استخدمت هذه العينات لقياس تراكيز جزيئات مناعية مختارة وهي IL-2, IL-6, IL-10, IFN-γ, MIF و CRP باستخدام ELISA kits ، كما وتم عمل أفلام الدم للقيام بعدّ الخلايا البيضاء التفصيلية.

أظهرت نتائج هذه الدراسة إمكانية التفريق بين مرضى الاكتئاب السريري المستجيبين لمثبطات استرجاع السيروتونين الانتقائية عن غير المستجيبين قبل البدء بالعلاج، بحيث أظهرت الدراسة فروقات واضحة بينهم من حيث تراكيز بعض الجزيئات المناعية و أعداد الخلايا. بشكل خاص، أظهر المستجيبون لمثبطات استرجاع السيروتونين الانتقائية تراكيز مرتفعة للجزيئات المناعية المقاسة في هذه الدراسة مقارنة بالتراكيز التي أظهرها غير المستجيبين لمثبطات استرجاع السيروتونين الانتقائية قبل البدء بالعلاج. كما وأظهر المستجيبون لمثبطات استرجاع السيروتونين الانتقائية عدد أكبر لخلايا lymphocytes وعدد أقل من خلايا neutrophils و eosinophils مقارنة مع غير المستجيبين لمثبطات استرجاع السيروتونين الانتقائية قبل العلاج. بعد العلاج، المستجيبون فقط لمثبطات استرجاع السيروتونين الانتقائية أظهراوا انخفاضاً في تراكيز بعض الجزيئات المناعية المقاسة في هذه الدراسة و عدد خلايا lymphocytes.

نتائج هذه الدراسة تعطي إشارة اولية في القدرة على معرفة إمكانية الاستجابة من عدمها عند مرضى الاكتئاب قبل البدء بالعلاج باستخدام لمثبطات استرجاع السيروتونين الانتقائية. لتأكيد هذه النتائج نحتاج وبلا شك للمزيد من العينات والمشاركين، لكن هذه أول دراسة تحاول التمييز بين مرضى الاكتئاب حسب استجابتهم للعلاج قبل بدئه. نجاح هذه الدراسة سيمكن الأطباء من اختيار العلاج المناسب، وسيختصر فترة من العلاج غير المجدي لدى مرضى الاكتئاب إن كانوا من غير المستجيبين لمثبطات استرجاع السيرتونين الانتقائية.

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