EVALUATION OF SERUM MIF LEVELS IN OBSESSIVE COMPULSIVE DISORDER PATIENTS: A CASE-CONTROL STUDY

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

School of Pharmacy
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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution
4. I/We have acknowledged all main sources of help.

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Approval

The project titled "Evaluation of Serum MIF Levels in Obsessive Compulsive Disorder Patients: A Case-Control Study" submitted by Syeda Tahsin Fahmida Humaira (22146068) of Spring, 2022 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy in April 2026.

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Ethics Statement

This study followed ethical rules set by the Declaration of Helsinki and the Bangladesh Medical Research Council (BMRC). Approval was taken from the Research Ethics Committee of the University of Asia Pacific before starting the study. All participants gave written consent after being clearly informed about the study's purpose, procedure, risks and benefits. The privacy and confidentiality of participants were carefully protected and they could leave the study at any time without facing any problem. Blood samples were collected safely by trained healthcare workers using proper clean techniques. The study followed both national and international ethical guidelines to ensure the safety and rights of all participants.

Abstract

Obsessive Compulsive Disorder (OCD) is a mental disorder where time consuming and disturbing unwanted obsessions and compulsions are observed. Various research has been done on the biological markers of OCD to determine a relation between these indicators and OCD. But none of the studies on the biomarkers has been able to demonstrate enough sensitivity and specificity and thus none are used for diagnostic studies till now. To help contribute in this field, this study was designed to find out the relationship of MIF in OCD. In this case-controlled study, 220 patients clinically diagnosed with OCD and 208 healthy controls from different regions of Bangladesh were enrolled. The serum samples were analyzed by using ELISA kit for the measurement of serum MIF. The serum MIF levels were significantly increased in patients with OCD compared with healthy controls. The findings of this study provide preliminary evidence of a possible association between serum MIF levels and OCD which may support the possible role of immune and inflammatory mechanisms in the pathophysiology of OCD.

Keywords: Obsessive Compulsive Disorder, Biomarkers, Cytokine, MIF, Neuroinflammation

Dedication

In loving memory of my father, Mir Tazrul Hossain, I dedicate this thesis to him. Though he is not here to witness this moment, his guidance, values and enduring love remain ever present. His strength and wisdom have been a constant source of inspiration throughout this journey. This accomplishment stands as a tribute to his memory with the hope that it would have made him proud.

I also dedicate this thesis to my beloved family and well-wishers whose endless love, support and encouragement have been my greatest strength throughout this journey. Their sacrifices, patience and belief in me have made it possible for me to reach this milestone.

Finally, this work reflects my own hard work, determination and perseverance through challenges and difficulties. Every step of this journey has taught me resilience, discipline and the value of never giving up.

This achievement stands as a symbol of collective support, personal effort and the hope for a better future ahead.

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List of Acronyms

OCD	Obsessive Compulsive Disorder
CSTC	Cortico-Striato-Thalamo-Cortical
GABA	Gamma-AminoButyric Acid
SAD	Systemic Autoimmune Disease
PANDAS	Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections
SSRI	Selective Serotonin Reuptake Inhibitor
SERT	Serotonin Transporter
GWAS	Genome Wide Association Studies
CNS	Central Nervous System
WHO	World Health Organization
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
ICD	International Classification of Diseases
SCID-OCSD	Structural Clinical Interview for Obsessive Compulsive Spectrum Disorders
Y-BOCS	Yale Brown Obsessive Compulsive Scale
Y-BOCS-II	Yale Brown Obsessive Compulsive Scale 2nd Edition
BDNF	Brain Derived Neurotropic Factor
DBH	Dopamine β Hydroxylase
MDA	Malondialdehyde
MIF	Macrophage Migration Inhibitory Factor
HPA	Hypothalamic–Pituitary–Adrenal
SLE	Systemic Lupus Erythematosus
RA	Rheumatoid Arthritis
ERK	Extracellular Signal-Regulated Kinase
PI3K	PhosphoInositide 3-Kinase
ELISA	Enzyme Linked Immunosorbent Assay
SPSS	Statistical Package for Social Sciences
BMI	Body Mass Index
ASD	Autism Spectrum Disorder

Chapter 1

Introduction

1.1 Obsessive Compulsive Disorder (OCD)

Obsessive Compulsive Disorder (OCD) is a mental disorder where time consuming and disturbing unwanted obsessions and compulsions are observed. Obsessions are interrupted and unwanted thoughts, desires and images and causes sudden stress, anxiety and a feeling that something is not right (Mahjani et al., 2021). Common obsessions are thinking that they are a bad person, feeling worried about hurting others or feeling contaminating others or themselves. Compulsions are repeated actions or mental habits like checking things, frequently cleaning, repetitive counting or arranging items. Compulsions are done to reduce stress and anxiety or to stop something bad from happening that they think because of their obsessive thoughts (Mahjani et al., 2021).

1.2 Epidemiology

According to the World Mental Health Surveys done on ten countries, about 4 out of every 100 people have experienced OCD at some point in their life and about 3 out of every 100 people had OCD within the past year (Stein et al., 2025). Females have slightly higher rates of OCD during adolescence and adulthood but in childhood males are often affected (Mathes et al., 2019).

OCD usually starts early in life, more than 80% of people develop it before early adulthood (Stein et al., 2025). In the general population of the ten countries studied, only 2.7% are severe, 22.9% are moderate, 47% are mild and 27.5% are very mild (Stein et al., 2025). At a national level, the Bangladesh National Mental Health Survey (2018-2019) found that about 0.7% of adults in Bangladesh have OCD (Bangladesh Association of Psychiatrists, 2021).

Most people with OCD do not get treatment and access to care is much better in wealthier countries than in poorer ones (Stein et al., 2025). People with OCD have a higher risk of death from both natural and unnatural causes than the general population. They are also more likely to have suicidal thoughts and about 14.2% have attempted suicide at some point in their lives (Mahjani et al., 2021).

1.3 Etiology

The causes of OCD are composites of many factors including genetic predisposition, neurobiological structure and functioning, changes in neurochemical function and imbalance of neurotransmitters, psychological condition and experiences, environmental condition and experiences. There is no single cause of OCD and it is thought to be a heterogenous disorder developed from the cumulative and interactions of these factors.

1.3.1 Genetic Factors

Several genomic research found that OCD has a strong genetic basis and is commonly run in families (Blanco-Vieira et al., 2023). Studies found that heritability of OCD is around 50% and first-degree relatives of OCD patients have a 4 to 8 times higher risk of developing the disorder compared to the general population (Strom et al., 2024).

Recent large scale twin studies show that identical twins are more likely to both have OCD than fraternal twins (Mataix-Cols et al., 2024), which indicates that OCD has a strong genetic influence. There is no single gene responsible for OCD, instead it is associated with multiple genes where multiple genes contribute a small amount to the risk of developing the disorder (Taylor, 2013).

1.3.2 Neurobiological Factors

OCD is highly correlated with a particular brain network known as the cortico-striato-thalamo-cortical (CSTC) circuitry. The CSTC loop is involved in impulse control and modulates compulsivity (Ting & Feng, 2011). The CSTC circuitry uses several neurotransmitters especially glutamate and gamma-aminobutyric acid (GABA) to modulate the flow of information within the CSTC circuitry and imbalances between these transmitters may generate the cardinal symptoms of OCD (Pittenger et al., 2011).

Various neuroimaging studies have demonstrated both functional and structural changes in the CSTC circuit of OCD patients (Alturaiqi et al., 2025). Increase in activity in these regions can be

correlated with the severity of obsessive and compulsive symptoms and research indicates that successful treatment of OCD can normalize these abnormal brain activity (Pittenger et al., 2011).

1.3.3 Cognitive Factors

The Cognitive Behavioral approach to obsessive compulsive disorder (OCD) represents the leading theory of a psychological explanation for OCD which demonstrates that people develop OCD because of faulty thinking and the misinterpretation of intrusive thought based on belief systems (Piras et al., 2022).

Everybody has OCD-like symptoms at some point because there are typically many common fairs to belief or rationale. So, the difference between having just regular OCD-like symptoms and having OCD itself occurs when that individual goes through a step-by-step process of concluding that the original symptom was indicative of something more serious and that they need to do something as an effort to combat this (Abramowitz et al., 2009). Most people will perform certain repetitive behaviours as a way to relieve themselves of their anxieties, but this hampers their ability to see that the anxiety they are experiencing is not real (Kalanthroff & Wheaton, 2022).

1.3.4 Environmental Factors

Different environmental factors have been identified to contribute to the development of OCD including childhood trauma, neonatal complication, brain injury and occurrence of stressful events (Raposo-Lima & Morgado, 2020). Stressful life events have been extensively researched as one of the main environmental risk factors for OCD (Brander et al., 2016). Major life stressors such as academic or family stress or illness and traumatic experiences such as abuse or accidents have been shown to either initiate the onset of OCD or to increase the severity of OCD symptoms (Brander et al., 2016).

The development of OCD can also result from a combination of psychosocial factors and neurobiological influences (Borrego-Ruiz & Borrego, 2025). Among these factors early life stress and childhood trauma in addition with family dynamics like overprotective or critical parenting

and cultural context like beliefs, norms and expectation are found to be relevant contributors (Borrego-Ruiz & Borrego, 2025).

In a large study of 1001 OCD patients, 60.5% patients had reported experiencing at least one major stressful event in their life such as the loss of a loved one, significant changes in interpersonal relationships and major emotional or financial problems with their family (Imthon et al., 2020).

1.3.5 Immunological Factors

OCD is linked to various immune system irregularities such as neuroinflammation, altered cytokines and immune cells (Coelho et al., 2024). Studies on OCD patients have shown increased quantities of pro inflammatory cytokines such as IL-1β, IL-6, IL-8, and TNF-α (Rodríguez et al., 2017).

Neuroinflammatory processes and immune cell activity such as microglial activation may influence neural circuits especially related to emotion and behavior involved in OCD (Coelho et al., 2024). Inflammatory signaling plays a major role in the disease development and thus targeting the immune system could improve patient outcomes (Bhatt et al., 2024).

Statistical studies indicate that people who have systemic autoimmune diseases (SADs) were more likely to experience OCD in their life as research demonstrates that patients with a history of SAD have an 1.85 times greater risk for developing OCD (Wang et al., 2018). Along with autoimmune diseases infections may trigger OCD in children and adolescents. One such example is a phenomenon called PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections) in which antibodies produced in response to an infection cause inflammation of brain tissue leading to the sudden development or worsening of OCD symptoms (Wang et al., 2018).

1.3.6 Perinatal Factors

Adverse pregnancy or childbirth events have been shown to significantly increase the risk of developing OCD regardless of any inherited factors shared amongst family members. There are

several perinatal risk factors associated with this increased incidence including maternal smoking (especially if she was smoking 10 or more cigarettes daily), breech presentation, cesarean delivery and preterm delivery (Brander et al., 2016).

There appears to be a dosage-response relationship wherein there are greater chances of developing OCD when comparing shorter gestational age, lower weight at the time of birth and the total number of perinatal complications. These complications could impact fetal brain development or interfere with the body's serotonin system that increases the chances of developing OCD later in life (Brander et al., 2016).

1.4 Symptom dimensions of OCD

OCD can present in multiple ways but is often defined according to primary symptom dimensions. Many individuals do not only experience one category of symptoms but usually experience multiple categories that overlap with each other from slightly to a greater extent.

1.4.1 Checking

Checking is one of the most common OCD compulsions that often involves doubt and need for certainty. It is a repetitive compulsive behavior usually done to prevent mistakes, harm or negative outcomes. At least for some time in their life, around 80% of OCD patients experience compulsive checking (Guo et al., 2025).

An observed trend of excessive checking of items has also appeared in threatening and neutral situations indicating that even minor amounts of uncertainty can result in compulsive behavior (Strauss et al., 2020). Checking is due to a patient's inability to trust their sensory processes and causes patients to want to exert conscious control over what typically occurs automatically or habitually (Strauss et al., 2020).

Patients with compulsive checking behavior may exhibit reduced confidence in their memories and perceptions but do not have actual memory deficits and may suffer from impaired higher level cognitive functioning (Dar et al., 2022). Checking is meant to reduce anxiety and uncertainty but actually it maintains or increases doubt which reinforces compulsions and creates a cycle (Guo et al., 2025).

1.4.2 Mental Contamination

Mental contamination is an internal feeling of dirtiness that occurs without direct physical contact with a contaminant. It comes from thoughts or internal experiences and is different from contact contamination which is caused by external substances (Miller et al., 2023). Factors like intrusive thoughts, memories of betrayal or trauma and interpersonal violation initiates mental contamination. It is often found to be associated with childhood trauma which mediates relationship between trauma and OCD symptoms (Corkish & Yap, 2024).

Intrusive thoughts or images can function like real contaminants which evokes feelings of dirtiness. (Coughtrey et al., 2015). The experience of mental contamination is typically related to intense emotional distress, feeling of disgust, shame and guilt. The internal contaminant sensations are not caused by physical dirt, and thus they tend to diffuse rather than localize to specific parts of the body (Coughtrey et al., 2012). In response to impure feelings patients often engage in compulsive behaviors such as excessive washing, avoidance and mental neutralization techniques (Coughtrey et al., 2012).

1.4.3 Symmetry

The symmetry dimension of OCD is characterized by a constant need for balance, alignment and exactness (Vellozo et al., 2021). There is a strong compulsion to arrange the objects or the actions until they feel accurate. A main characteristic of symmetry is having exactly the right experiences which is a feeling of incompleteness or internal discomfort until actions or arrangements meet a standard for correctness (Fornés-Romero & Belloch, 2017).

Visual asymmetry or misalignment in the surroundings and sensory irregularities typically triggers symmetry symptoms that creates a feeling of having an imbalance or incompleteness. Patients satisfy the discomfort of asymmetry by engaging in repetitive behaviors such as arranging, ordering or repeating an action until a sense of completeness is achieved (Vellozo et al., 2021).

In a large-scale study of 1001 patients, symmetry dimension was the most prevalent (86.8%) and common signs were perfectionistic concerns (73.4%), checking for errors (66.4%) and ordering habits (61.6%) (Vellozo et al., 2021).

1.4.4 Intrusive Thoughts

Intrusive thoughts are unwanted involuntary mental events that are often distressing, recurring and do not comply with personal values (Hinuma et al., 2025). Cognitive distortions such as beliefs of having a thought is morally equivalent to acting on it, contribute to the distress related with intrusive thoughts by making patients interpret them as dangerous or morally significant (Kim & Lee, 2020).

Intrusive thoughts commonly provoke intense emotional reactions including fear, anxiety, shame and guilt which leads to the compulsive neutralizing behavior (Hinuma et al., 2025). Common themes of intrusive thoughts are fears of harm, moral or religious misdeed and sexual or taboo content which violates personal values and increases distress (Hinuma et al., 2025).

1.5 Pathophysiology

1.5.1 Cortico-Striato-Thalamo-Cortical (CSTC) Circuit Dysfunction

The Cortico-Striato-Thalamo-Cortical (CSTC) model is a neurobiological model that helps understand why we develop OCD. This model describes how different regions of this circuit communicate to control the thoughts, develop habits and inhibit impulsive actions. The CSTC

models include multiple neural pathways that are communication loops that begin at the frontal cortex and then go to the striatum, to the thalamus and back to the cortex (Jalal et al. 2023).

CSTC circuits that are overactive and have poor connectivity can be the reason behind undesirable thoughts and behaviors and repetitive actions. Obsessive-Compulsive Disorder (OCD) is also believed to originate in neural circuits of the CSTC model, as many neuroimaging studies of anterior thalamus, striatum, and thalamus, along with the orbitofrontal and anterior cingulate cortices, show pathological/abnormal activation of these circuits (Goodman et al. 2021). For example, studies have found reduced functional connectivity within CSTC loops in unmedicated OCD patients compared with healthy controls (Posner et al., 2013)

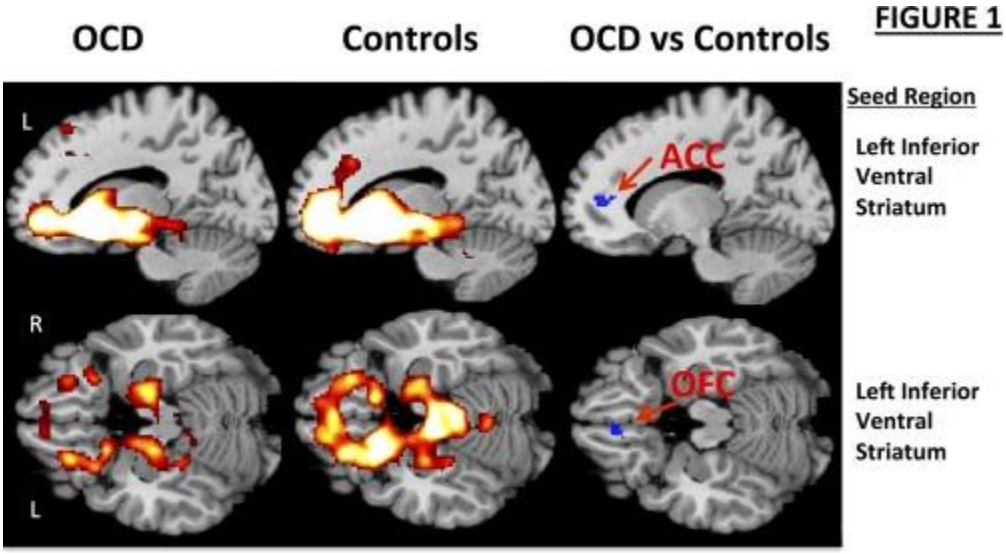

Figure 1: Resting state functional connectivity MRI of OCD patients vs healthy controls (Adapted from Posner et al., 2013)

Figure 1 illustrates the neurocircuitry involved in OCD which is adapted from Posner et al., 2013 for the better demonstration of pathophysiology of OCD.

OCD also does not appear to be limited to a single neural circuit as there is significant evidence of the presence of neural circuit abnormalities in multiple OCD patients. These include the affective circuit that contributes to emotion processing, cognitive circuits that are responsible for planning

and executive control and sensorimotor circuit which has been increasingly related with compulsive habit formation (Jalal et al., 2023).

1.5.2 Neurotransmitter Dysregulation

Dysregulation of key neurotransmitter systems such as glutamatergic, serotonergic and dopaminergic systems is thought to be the reason behind the functional disturbance observed within the CSTC circuits in OCD. The relationship between OCD and the serotonergic system has been well established because the OCD patients have positively responded to Selective Serotonin Reuptake Inhibitors (SSRIs).

Since serotonin modulates mood, inhibition and anxiety, dysfunction may impair suppression of intrusive thoughts (Goodman et al., 2021). Molecular imaging studies demonstrated that serotonin transporter (SERT) binding potential was decreased in midbrain, brainstem and thalamus. This decrease is in line with the serotonergic dysfunction in patients suffering from OCD (Pastre et al., 2025).

Changes in dopaminergic activity within frontostriatal pathways may contribute to the development of OCD. An excess of dopamine in the striatum may enhance habits. An imbalance of dopamine in brain areas like the ACC may lead to rigid thinking and problems to notice mistakes (Mota et al., 2026).

Excessive excitatory signaling may result from glutamatergic dysregulation in the striatum. The enhanced excitatory activity is thought to be a contributing factor to the development of intrusive thoughts and repetitive behaviors (Karthic et al., 2020).

1.5.3 Genetic and Neurodevelopment Factors

It has been shown through family and twin studies that the heritability of OCD is significant (Blanco-Vieira et al., 2023). Genome wide association studies (GWAS) have shown that OCD is a very polygenic disorder involving thousands of genetic variants with a small effect on overall risk (Strom et al., 2025). According to the finding of a large scale GWAS, many risk loci and

candidate genes have been identified. Many of these genes are expressed in various brain regions including the cortex and hippocampus and are associated with dopaminergic and glutamatergic signaling pathways (Strom et al., 2025).

Studies revealed that OCD has a genetic link with other psychiatric disorders such as anxiety disorders, major depression and Tourette syndrome (Strom et al., 2025). Genetic risk factors may affect early brain maturation because gene expression and neural circuit formation are significantly influenced by development age. These effects are especially important in circuits related with cognitive control and habit formation and increases vulnerability to OCD (Shephard et al., 2021).

1.5.4 Neuroinflammatory and Autoimmune Hypotheses

Recent research has increasingly related immune dysregulation and neuroinflammatory processes in the pathophysiology of OCD. Inflammatory related signaling pathways have been found to have considerable effect on the condition (Bhatt et al., 2023). Studies have found differences in pro inflammatory cytokine levels and immune cell proliferation and activity in OCD patients compared with healthy controls (Coelho et al., 2024).

Recent neuroimaging studies have demonstrated neuroinflammatory findings such as microglial activation in patients with OCD which supports the fact that there is involvement of inflammatory processes in the development of OCD (Liu et al., 2025).

Microglia are the main immune cells in the central nervous system (CNS) and are important for the formation of synapses, development of neural circuits and regulation of behavior. They represent a possible reason for the immune dysregulation to affect circuitry in the brain associated with OCD (Zhu et al., 2023).

1.5.5 Cognitive and Behavioral Neurobiological Models

Cognitive and behavioral neurobiological models of OCD focus on the interaction between dysfunctional neural circuits and maladaptive cognitive processes that lead to the characteristic

symptoms. Dysfunction within the CSTC circuitry is the main focus of this model (Ting & Feng, 2011).

From a cognitive view, people with OCD show deficits in execution skill such as having poor inhibitory control and cognitive flexibility and these deficits would be factors that contribute to the continuation of unwanted thoughts and repetitive behaviors (Abramovitch et al., 2013). Additionally, a great deal of research has demonstrated increased error monitoring where patients show hyperactive brain activity in response to errors which could foster compulsive checking and correcting (Hajcak & Simons, 2002).

Behaviorally, OCD is increasingly being thought of as being a result of poor goal directed control coupled with over excessive habit formation, resulting in continued compulsion despite their lack of any adaptive value (Gillan et al., 2016).

1.6 Diagnosis

1.6.1 Diagnostic Criteria

OCD is diagnosed using standard guidelines of major classification systems like DSM-5 and the World Health Organization (WHO). Obsessions are repeated thoughts, urges or images that come into mind again and again. These are unwanted and intrusive and usually cause strong anxiety and distress. Compulsions are repeated actions or mental activities that a person feels forced to do because of an obsession or strict rules. Compulsive actions are usually done to reduce anxiety or to prevent something bad from happening (Leckman et al., 2010).

A significant amount of time for example over one hour per day must be spent on obsessive and compulsive thoughts or behaviors in order to be diagnosed as OCD. It must cause serious distress or problems in daily life such as in work, social activities or other important areas to be clinically significant. Symptoms cannot be caused by drugs, medication or another medical condition and they cannot be explained better by a different mental health disorder (Leckman et al., 2010).

The DSM-5 also describes different levels of insight in OCD. People may have good or fair insight where they know their beliefs are unreasonable, poor insight and no insight or delusional beliefs where they are convinced their beliefs are true (Leckman et al., 2010).

1.6.2 Clinical Interview and Structured Assessment

A clinical interview is a conversation between a clinician and a patient to learn about symptoms, history and daily struggles of the patient caused by OCD. During this process the clinician will explore the patient's obsessions and compulsions as well as gather additional relevant information regarding how these symptoms manifest over time. The interview can be unstructured like free from questions and structured or semi structured that follows specific guidelines from DSM-5 (Rapp et al., 2016).

Structured and semi structured interviews are more reliable and consistent than unstructured ones. During this clinical interview, the clinicians will reference the DSM or ICD as part of the process to evaluate the patients OCD related symptoms within the context of the evaluation process. Clinicians must attempt to limit their own biases during the evaluation process and also ensure that they appropriately assess the primary aspects of OCD. (Lochner et al., 2024).

One specific tool available to evaluate patients for OCD is the recently developed SCID-OCSD. This is a structured clinical interview that has been specifically developed to assess patients with OCD related disorders with an opportunity to evaluate potential comorbid disorders like body dysmorphic disorder and skin picking disorder that could impact treatment planning. Structured interviews like SCID-OCSD make it easier to detect these related conditions which might be missed in general assessments (Lochner et al., 2024).

Structured clinical interviews also have their limitations as they can require one to three hours to conduct which may be tiring for both clinicians and patients. Clinicians also must gather information from multiple sources like parent and children and include information gathered through their own observation in their final evaluation (Rapp et al., 2016).

1.6.3 Severity and Clinician rated scales

Once an individual has been diagnosed with OCD, it is necessary to determine how serious their symptoms are. Symptom severity is useful for developing a treatment plan and monitoring progress through therapy to see if the treatment is successful. Clinician rated scales are useful because they provide a standard and reliable way to measure seriousness of the symptoms and their effect on daily life (Castro-Rodrigues., 2018).

The most widely used tool for measuring the severity of OCD in both clinical and research settings is the Yale Brown Obsessive Compulsive Scale (Y-BOCS). The Y-BOCS classifies important components of obsessions and compulsions such as how long they take, the level of distress they create, how they affect daily functioning, how much a person is able to resist them and how much control they feel they have over them (Castro-Rodrigues., 2018).

The Y-BOCS is considered to be a very reliable and valid tool for understanding the severity of OCD and is commonly thought of as the gold standard to assess the severity of OCD. The scale is able to show if there has been any improvement in a person's OCD symptoms after receiving therapy (Castro-Rodrigues., 2018).

An updated version called Y-BOCS-II was developed to further improve the rating scale's ability to evaluate OCD symptom severity in OCD patients. The Y-BOCS-II includes better structured questions as well as the inclusion of avoidance behaviors which were previously not represented very well in the original Y-BOCS scale. Research shows that Y-BOCS-II is more accurate and reliable and stays consistent with the original scale (Castro-Rodrigues., 2018).

1.7 Biomarkers in OCD

Biomarkers in OCD are being studied to give clear and objective ways to help with diagnosis, predicting outcomes and tracking treatment response. Research shows that OCD is linked to changes in several biological systems. These include imbalances in neurotransmitters, changes in

brain growth and support signals, dysfunction in the immune system and increased oxidative stress (Sultania et al., 2024).

Neurotropic biomarkers like Brain Derived Neurotropic Factor (BDNF) have effects on brain cell survival and signaling and have been identified as a potential biomarker for OCD. Research finds significantly lower levels of BDNF in OCD patients compared with healthy controls (Hao et al., 2022). Dysregulation of neurochemicals such as serotonin and dopamine plays a central role in the brain circuit related to OCD. Research suggests dopamine β hydroxylase (DBH) as a potential biomarker for OCD (Sultania et al., 2024).

Research finds causes and underlying mechanisms of OCD are closely linked to inflammatory biomarkers and suggest that future research should explore effectiveness of anti-inflammatory treatments in the early stages of the disorder (Alsheikh & Alsheikh, 2021). Evidence found increased oxidative stress in OCD patients which can contribute to neuronal damage. Studies suggest Malondialdehyde (MDA) as a marker of lipid peroxidation in OCD patients (Sultania et al., 2024). Although many findings are promising, no single biomarker is currently useful in clinical practice for OCD. Among the different potential markers, immune related markers like cytokines are getting more attention and need further study.

1.8 Cytokines role in OCD

Research suggests that OCD may involve not only neurotransmitter changes but also dysfunctions in the immune system. Proinflammatory cytokines which are small proteins involved in immune and brain inflammation may play a role in OCD. Specifically, IL-1β and IL-6 can activate brain immune cells such as microglia and astrocytes and interfere serotonin, dopamine and glutamate signaling pathways which could potentially contribute to OCD symptoms (Gray & Bloch, 2012).

Recent studies find increased levels of serum IL-1β and IL-6 levels in OCD patients compared to healthy controls and suggests that these cytokine levels in blood samples may be used as early indicators to assess the risk of developing OCD. They also found a significant positive relationship

between the cytokines studied and Y-BOCS scores which indicates that higher inflammation is associated with more severe OCD symptoms (Sharmin et al., 2024).

Cytokines may also be relevant to treatment outcomes in OCD. Some studies suggest that inflammation can influence response to SSRIs which are the first line medications for OCD. People with higher levels of inflammatory markers like cytokines may poorly respond or have more treatment resistance. Conversely, those who improve with treatment may also show a decrease in these inflammatory markers. This suggests that immune system activity may help explain differences in treatment response in OCD (Bhatt et al., 2023).

Overall, abnormal cytokine levels suggest that the immune and inflammatory system plays a significant role in OCD. However, results are not consistent across studies and more long-term research is needed to understand cause and effect and possible therapeutic implications.

1.9 MIF

Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine that plays an important role in many cellular activities, inflammatory response, immune response, apoptosis and counter- regulation of glucocorticoids (Sumaiya et al., 2022). At first, MIF was identified as activated T cells generated cytokines but recently it has been recognized as a multifunctional key cytokine that is secreted by many other immune and non-immune cells (Sumaiya et al., 2022). When cells are exposed to pro-inflammatory cytokines, microbes or specific antigens, MIF is released and it acts through both autocrine and paracrine signaling that leads to the production of pro-inflammatory cytokines (Bloom & Al-Abed, 2014).

MIF binds to CD74, CD44, CXCR2, CXCR4 and CXCR7 and activates different pathways that result in activation of proinflammatory genes (Vázquez et al., 2023). When secreted by neuroendocrine and immune tissues, MIF acts as a glucocorticoid antagonist within the immune system and functions as a neuroendocrine mediator that regulates multiple endocrine circuits (Fingerle-Rowson & Bucala, 2001).

MIF has potential parts in CNS disorders as they are widely expressed in the CNS (Zhang et al., 2024). Research suggests that elevated plasma levels of MIF are related with dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis and can cause depressive symptoms in patients (Bay-Richter et al., 2015).

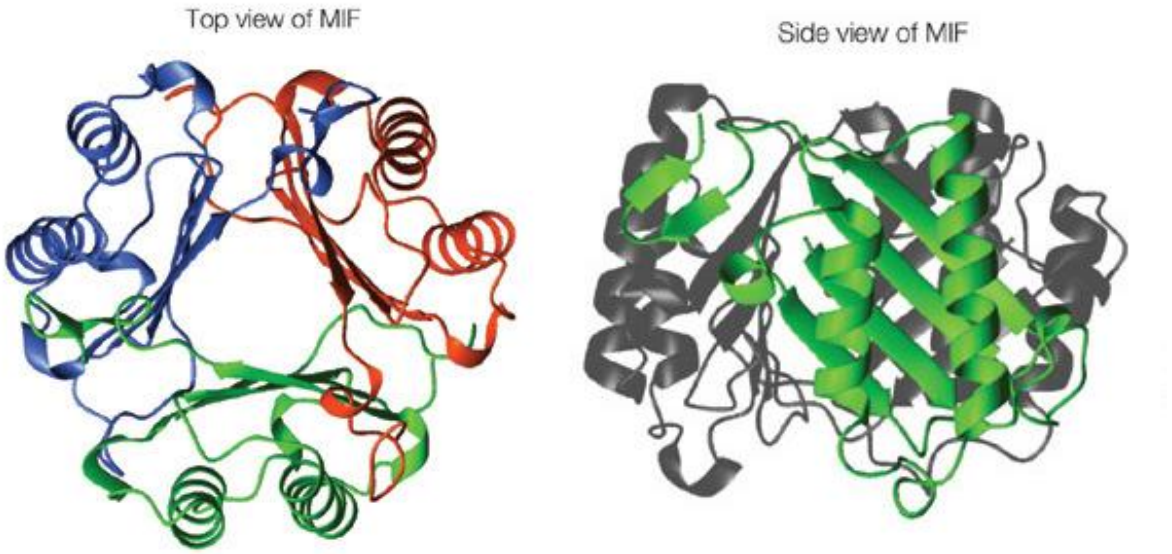

Figure 2: 3D structure of MIF (Adapted from Calandra & Roger, 2003)

Figure 2 presents the three-dimensional structure of MIF, adapted from Calandra & Roger (2003) to enhance visualization of the structure of the molecular configuration.

1.9.1 Biological Roles of MIF

MIF has been identified as a key regulator of both innate and adaptive immunity. When the body is exposed to stress and different inflammatory stimuli, it rapidly releases MIF from multiple immune cells including macrophages, T cells and dendritic cells (Calandra & Roger, 2003). It increases the body's immune response by promoting antigen presentation, cytokine production and macrophage activation (Meza et al., 2025).

MIF exhibits its potent pro-inflammatory function by inducing the production of other cytokines including TNF, IL-6 and IL-1 groups cytokines (Lang et al., 2018). A unique feature of MIF is that it can surpass the immunosuppressive effects of glucocorticoids by reversing glucocorticoid induced inhibition of cytokine synthesis (Calandra & Roger, 2003).

MIF also induces cell multiplication and inhibits apoptosis of cancer cells. When researchers reduced MIF levels, cell multiplication of cancer cells was inhibited and apoptosis of cancer cells was induced (Guo et al., 2015). MIF also functions in a similar way to chemokines when promoting the migration and recruitment of leukocytes into inflammatory and infection sites (Grieb et al., 2010).

MIF is expressed in different parts of CNS including astrocytes, neurons and microglia and regulates neuroinflammatory responses. It also participates in neuronal stem cell proliferation, apoptosis regulation and neuroplasticity regulation (Zhang et al., 2024). Researchers suggest that other than neuroinflammatory regulation, MIF has more functional importance that needs to be explored.

1.9.2 Clinical Roles of MIF

MIF is found to be related in the pathogenesis of autoimmune diseases like systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) where increased levels are associated with increased disease severity. Researchers suggest that MIF inhibitors might provide effective therapeutic effects (Bilsborrow et al., 2019).

In diseases like sepsis, where infection causes inflammation in the systemic circulation, studies have found a significant level of elevated MIF level in blood and thus suggests that MIF can be a valuable diagnostic biomarker in sepsis (Toldi et al., 2021). It is also overexpressed in several cancer types and their level of expression indicates a worse sign for the patients. Studies find trustable results when MIF is inhibited by nanobodies, antibodies or small synthetic molecules (Barthelmess et al., 2023). Recent studies found MIF to be a potential biomarker for depression and anxiety risk (Lipschutz et al., 2018).

1.9.3 Mechanism of Action of MIF

MIF works by first binding to a receptor called CD74 which acts as the main receptor that starts it signaling pathways (Nothnick et al., 2018). Binding with CD74 leads to promoting cell growth, cell cycle arrest and apoptosis (Calandra & Roger, 2003).

MIF shows its function as an inflammatory mediator similar to chemokine by binding to CXCR3 and CXCR4 that leads to leukocyte accumulation. It also binds with CD74/CXCR2 complexes to recruit inflammatory cells (Schwartz et al., 2009).

After receptor binding, MIF activates different signaling pathways including phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) and extracellular signal-regulated kinase (ERK) (Xie et al., 2026). By activating these pathways MIF exhibits different functions including rebuilding tissues and inhibiting the tumor suppressor p53. It acts as a growth factor by promoting cell survival and multiplication of different cells (Song et al., 2022).

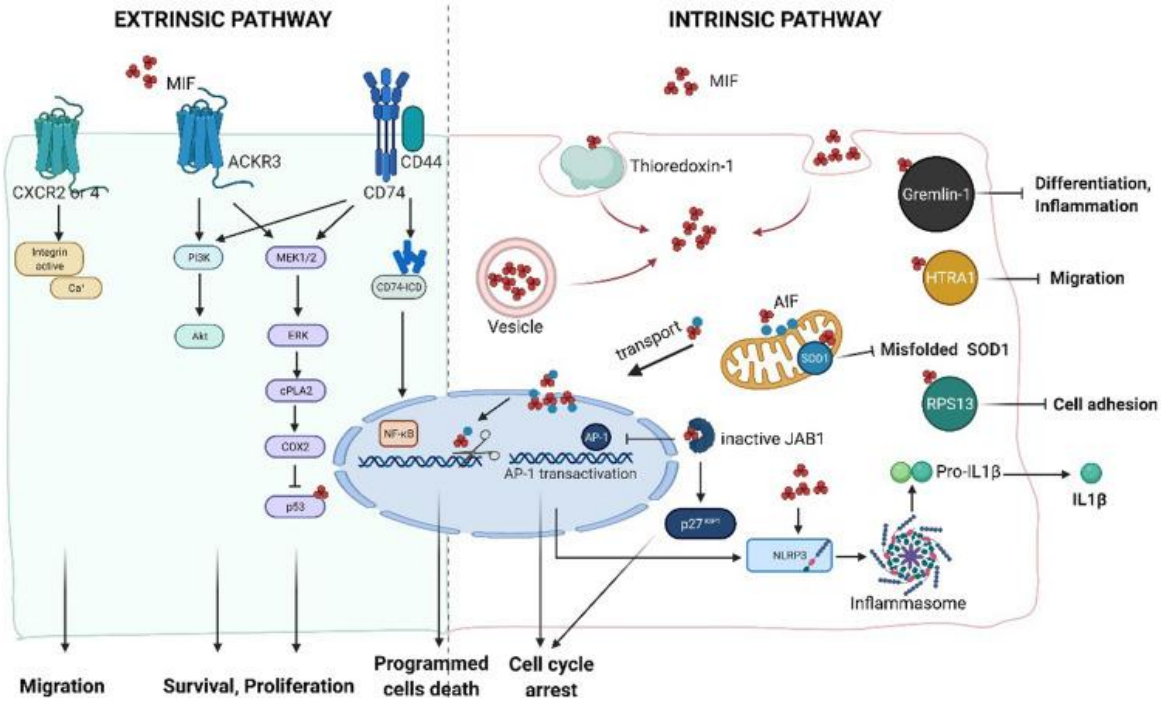

Figure 3: Signaling pathways of MIF (Adapted from Song et al., 2022)

Figure 3 illustrates the extrinsic and intrinsic signaling pathways of MIF which is adapted from Song et al., 2022 for better demonstrating the mechanism of action through different binding action.

1.9.4 Possible role of MIF in OCD

Research shows that proinflammatory cytokines may play a role in how OCD develops (Gray & Bloch, 2012). Proinflammatory cytokines can cause neuroinflammation, reduce neuroplasticity, disrupt neurotransmitter functions and thus participate in the pathophysiology of multiple mental disorders including OCD (Sarker et al., 2023).

Proinflammatory cytokines specifically interleukin-1β (IL-1β) and interleukin-6 are found to take part in pathophysiology of OCD by activating microglia and astrocytes that leads to disruption of neurotransmitter pathways (Sarmin et al., 2024). Elevated interleukin-17 (IL-17) level is also demonstrated to be involved in inflammatory pathways and their positive association with severity of OCD (Sarker et al., 2023).

MIF regulates inflammatory responses and can cause the production of multiple cytokines (Bay-Richter et al., 2015), which suggest that it can play a role in cytokine mediated neuroinflammation which occurs in OCD. MIF also regulates stress response of the HPA axis and has been associated with stress reactivity and anxiety (Lipschutz et al., 2018), both of these are key parts in the pathophysiology of OCD. The involvement of MIF in psychiatric disorders supports the fact that it may have an influence on behavior and emotional regulation in OCD.

1.10 Rationale of the study

Obsessive-Compulsive Disorder (OCD) has been increasingly linked to immune dysregulation, altered stress reactivity and neuroinflammatory processes. The role of MIF is established in modulating these pathways in various studies. Therefore, there is strong biological rationale for the investigation of MIF activity in OCD. But there is no direct research study connecting MIF to OCD patients. Since there is evidence that MIF is related to other psychiatric conditions like depression and anxiety, the potentiality of MIF as a biomarker in OCD and their association with the severity of disease needs to be studied.

Chapter 2

2. Materials and Methods

2.1 Study Participants

In this case-controlled study, 220 patients clinically diagnosed with OCD were enrolled from National Institute of Mental Health, Bangladesh. For the control set, 208 healthy controls from different regions of Bangladesh were accurately compared by sex and age to match with OCD patients and were enrolled. All the participants were asked structured clinical questionnaires following the Y-BOCS scale to identify OCD severity. The healthy controls and OCD patients were matched by sex, age and socio demographic profile.

2.1.1 Inclusion Criteria

- Patients who were diagnosed by a certified professional psychiatrist were accepted to participate in the study
- Both male and female participants were accepted
- Volunteers between 18 to 60 years old were accepted
- Both married and unmarried participants were accepted
- Patients who had Y-BOCS score of 8 or higher were accepted in the OCD patient group
- Participants who gave their blood sample and other information with their consent

2.1.2 Exclusion Criteria

- Patients who had Y-BOCS score of 7 or less were rejected
- Participants who were not willing to participate in the study were rejected
- Patients with severe medical condition were rejected
- Patients with psychiatric disorders other than OCD were excluded
- Pregnant women were excluded
- Participants who had alcohol and drug habits were excluded

- Patients that were taking medications that could interfere with the concentration of MIF were excluded

2.1.3 Consent Form

All study participants provided their written consent before blood collection and data gathering. A consent form sample for the participants is given below:

সম্মতিপত্র

১.	এই গবেষণা কর্মের ধরণ, উদ্দেশ্য এবং পদ্ধতি সম্পর্কে কি পুরো জানতে পেরেছেন?	:	হ্যাঁ / না
২.	এই গবেষণায় নার্সদের মাধ্যমে রক্ত সংগ্রহ করা হবে, এ বিষয়ে কি অবহিত হয়েছেন?	:	হ্যাঁ / না
৩.	এই গবেষণার ফলাফল এবং সম্ভাব্য কল্যাণের বিষয়ে আপনার ধারনাটি স্পষ্ট হয়েছে কি?	:	হ্যাঁ / না
৪.	এই গবেষণা কর্মে অংশগ্রহণ, সহযোগিতাদান, অথবা বিরত থাকার সিদ্ধান্ত আপনি কি স্বাধীনভাবে গ্রহণ করতে পেরেছেন?	:	হ্যাঁ / না
৫.	গবেষণা কর্মে অংশগ্রহণের ফলে আপনার মৌলিক মানবাধিকার কি ক্ষুণ্ন হচ্ছে?	:	হ্যাঁ / না
৬.	আপনি কি পরিপূর্ণ ভাবে জ্ঞাত যে, আপনার তথ্যাবলির গোপনীয়তা বজায় রাখা যাবে?	:	হ্যাঁ / না
৭.	গবেষণা কর্মে অংশগ্রহণের জন্য আপনাকে কোন পারশ্রমিক অথবা ভ্রমণভাতা দেয়া হবে না, এ বিষয়ে কি যথেষ্ট অবহিত আছেন?	:	হ্যাঁ / না

অনুমতি পত্র

এই গবেষণা কর্মের উদ্দেশ্য, পদ্ধতি ও উপযোগিতা সম্পর্কে পূর্ণ ধারণা পেয়ে, এর নীতিগত বৈশিষ্ট্য সমূহের প্রতি আমার সম্মতি প্রকাশ করছি। গবেষণা কর্মে অংশগ্রহণের জন্য আমি কোন ব্যাক্তি বা গোষ্ঠী দ্বারা প্রভাবান্বিত হইনি অথবা আমার মৌলিক মানবাধিকার ক্ষুণ্ন হয়নি। অতএব, যথাযথ পর্যালোচনা সাপেক্ষে আমি স্ব-প্রণোদিত হয়ে এই অনুমতি পত্রে স্বাক্ষর করছি।

নাম: স্বাক্ষর

_____ _____

2.2 Blood Sample Collection

2.2.1 Materials required for collection of blood sample

Table 1: Materials required for collection of blood sample

Materials	Volume
Hexisol	250 mL
Disposable syringe	10 mL
Falcon tube	10 mL
Eppendorf tube	1.5 mL
Disposable butterfly needle	
Sterile cotton	
Ice bag	
Ice box	
Band aid	
Tourniquet	

2.2.2 Blood Sample Collection from Participants

Blood samples were taken from 220 OCD clinically diagnosed patients and 208 healthy controls. The cephalic vein of the participants was used to collect blood by using 10 mL of syringe and around 8 to 10 mL of blood was placed in the falcon tubes. Then the falcon tubes were kept at room temperature for about half an hour and then stored in the ice box for preservation.

2.2.3 Preparation of Serum and Storage

After collecting blood, the blood was allowed to clot in the falcon tubes. Then the blood was centrifuged at 3000 rpm for 15 minutes to separate the blood cells and serum. A clear yellowish part was separated as serum after centrifugation. Then eppendorf tubes were used to collect the serum by using a micropipette and stored in a -80°C refrigerator.

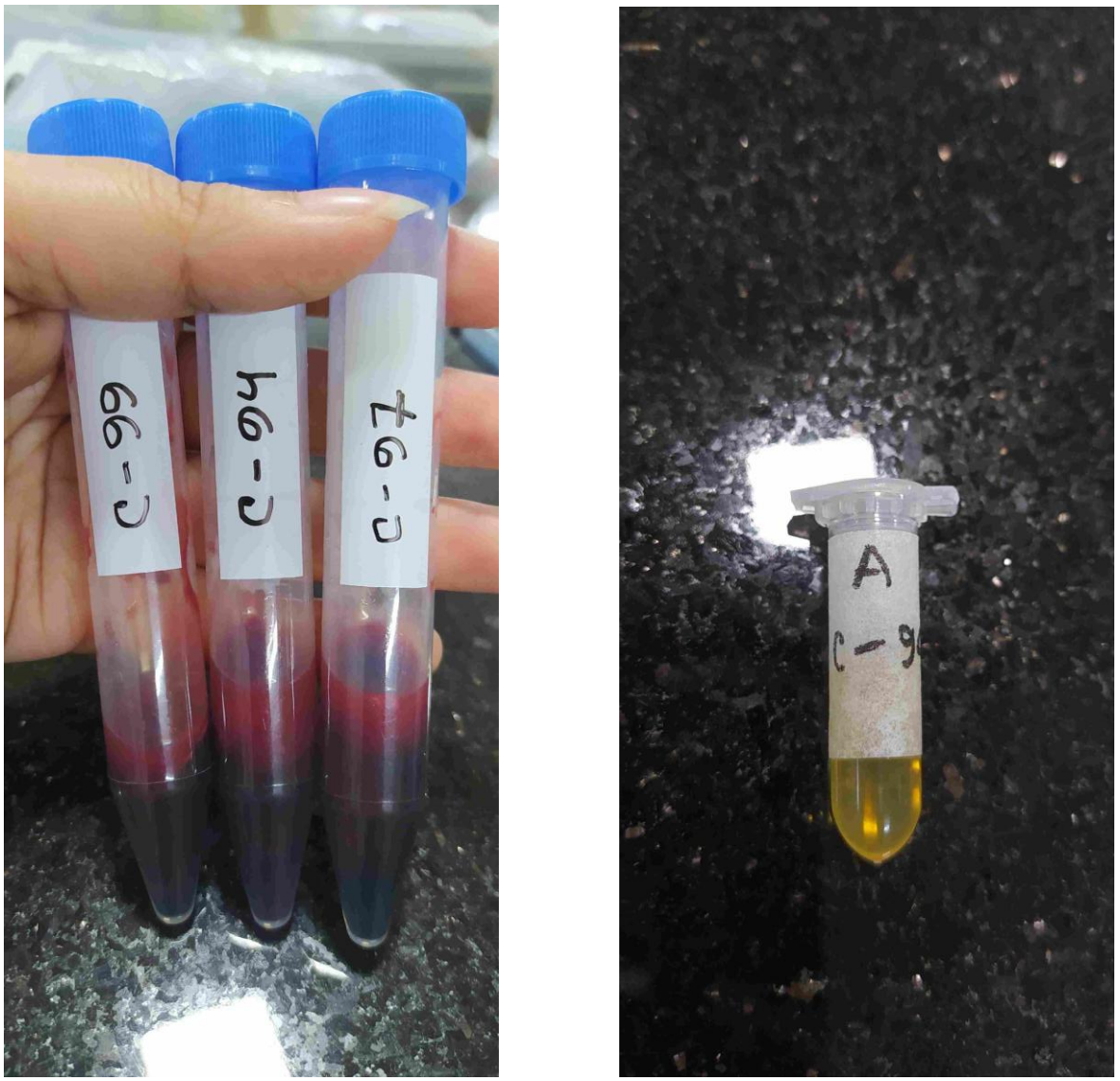

Figure 4: Collected Blood and Serum

2.3 Analysis of Sample

For the analysis of the samples of serum and measurement of the concentration of MIF, the enzyme linked immunosorbent assay (ELISA) kit was used.

2.3.1 Materials used for the analysis of MIF

To measure the concentration of serum MIF, Human MIF ELISA Kit EZ-Set™ (DIY Antibody Pairs) from Booster Bio, USA was used.

Table 2: Materials used for the analysis of MIF

Description	Quantity/Volume
Rabbit anti human MIF polyclonal antibody (Capture Antibody)	500 µl, 0.4 mg/mL
Biotinylated goat anti human MIF polyclonal antibody (Detection Antibody)	500 µl, 14 µg/mL
Avidin Biotin Peroxidase Complex (ABC)	500 µl
Lyophilized recombinant human MIF standard	10 ng/tube ×3
96 well removable strip microplate	5
Plate sealers	20
Reagent Diluent (10X)	20 mL/vial ×2
Stop solution	10 mL/vial ×5
Color Developing Reagent	10 mL/vial ×5
PBS (20X)	25 mL/vial ×2
PBS-T (20X)	25 mL/vial ×2

Figure 5: ELISA kit of Human MIF

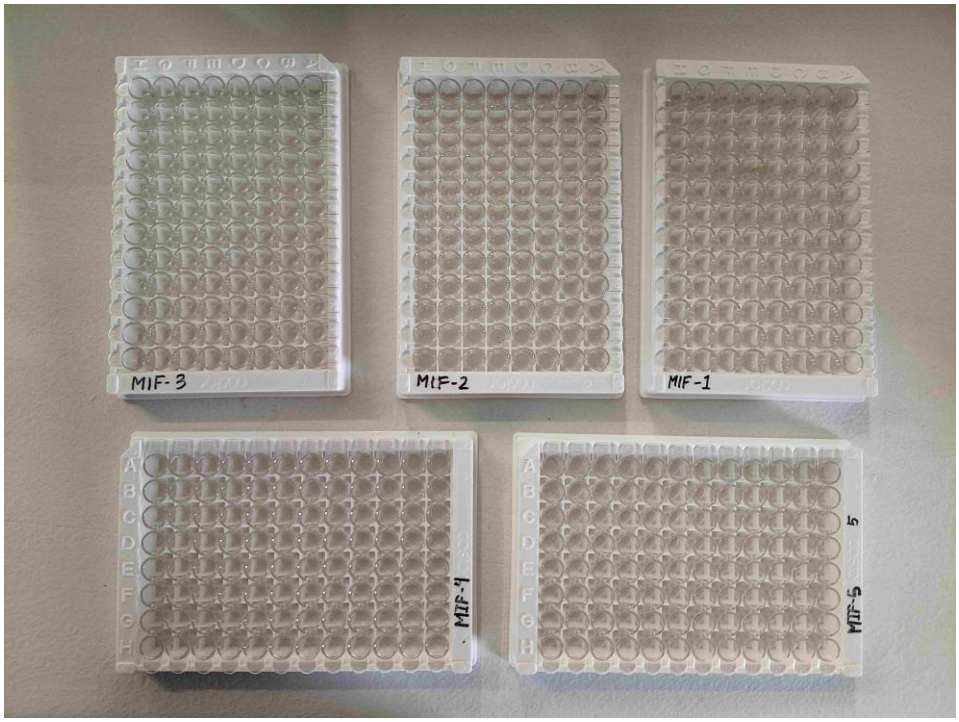

Figure 6: 96 well microplates

Figure 7: ABC, MIF detection antibody and capture antibody

Figure 8: MIF Standard and Stop solution

Figure 9: Reagent Diluent, PBS and PBS-T

Figure 10: Substrate solution (Color Developing Reagent)

2.3.2 Preparation Required for the Experiment

Before starting the experiment, all the reagents were kept at room temperature. All required solutions were freshly prepared before each step.

2.3.2.1 Plate Preparation

The diluted MIF capture antibody was used to coat microplates. Then each plate was sealed using plate sealer and put in an incubator for overnight incubation at 4°C. After incubation the liquid of the plate was discarded. Then reagent diluent was used to block each well and the plate was allowed to incubate at room temperature for two hours. After incubation each plate was washed using 300 µl of PBS for each well for three times.

2.3.2.2 Human MIF Standard Preparation

The MIF standard was prepared 2 hours before performing the experiment. At this stage, one 10 ng of lyophilized human MIF standard was used. The vial was firmly agitated before using it. The MIF standard was prepared in one milliliter of reagent diluent at a final concentration of 10 ng/ml. Before dilution was performed, the standard was allowed to sit for 10 minutes after the addition of reagent diluent. 7 eppendorf tubes were serially numbered from one to seven before dilution. The resulting concentrations of the standards are presented below:

- Tube 1 :1000 ng/mL
- Tube 2 :500 ng/mL
- Tube 3 :250 ng/mL
- Tube 4 :125 ng/mL
- Tube 5 :63 ng/mL
- Tube 6 :31 ng/mL
- Tube 7:16 ng/mL
- Tube 8 :0 ng/mL

2.3.2.3 Assay Procedure of MIF

1. All reagents and working solutions were prepared as directed.
2. In each well, 100 µl of the collected samples, controls and standards of different concentration were added.
3. Then the plates were covered with provided plate sealers and incubated for 90 minutes at 37°C.
4. After incubation, the cover was removed and the liquids in the wells were discarded into the appropriate place. The plate was inverted on a flat surface onto some tissue papers and tapped gently to remove any residual liquid. The whole process was carefully done so that the wells did not get completely dry at any point.
5. Then in each well, 100 µl of the freshly prepared Biotinylated goat anti human MIF polyclonal antibody was added.
6. Then the plates were covered with provided plate sealers and again incubated for 60 minutes at 37°C.
7. After 60 minutes of incubation, 300 µl of PBS was added to each well to wash the plates for 3 times.
8. After washing, 100 µl of the freshly prepared Avidin Biotin Peroxidase Complex was added into each well and again incubated for 30 minutes at 37°C.
9. After incubation the plates were washed 5 times with PBS-T and 300 µl of PBS-T was used for washing each well.
10. Then in each well, 90 µl of Color Developing Reagent named substrate solution was added and incubated for around 25 to 30 minutes at 37°C.
11. Then in each well, 100 µl of stop solution was added and the color was observed for an immediate change to yellow.

12. The absorbance of the prepared plates was measured using a microplate reader at a wavelength of 450 nm within 30 minutes after the reaction had stopped.

Figure 11: While adding stop solution color change was observed

Figure 12: After adding stop solution yellow color was developed

2.4 Microplate Design for the Assay Procedure

Five 96 well microplates were used for the analysis of MIF. The plate designs are given below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std.1	Std.2	Std.3	Std.4	Std.5	Std.6	Std.7	B	P1	P2	P3	P4
B	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16
C	P17	P18	P19	P20	P21	P22	P23	P24	P25	P26	P27	P28
D	P29	P30	P31	P32	P33	P34	P35	P36	P37	P38	P39	P40
E	P41	P42	P43	P44	C1	C2	C3	C4	C5	C6	C7	C8
F	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20
G	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30	C31	C32
H	C33	C34	C35	C36	C37	C38	C39	C40	C41	C42	C43	C44

Figure 13: Plate 1 Design

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std.1	Std.2	Std.3	Std.4	Std.5	Std.6	Std.7	B	P45	P46	P47	P48
B	P49	P50	P51	P52	P53	P54	P55	P56	P57	P58	P59	P60
C	P61	P62	P63	P64	P65	P66	P67	P68	P69	P70	P71	P72
D	P73	P74	P75	P76	P77	P78	P79	P80	P81	P82	P83	P84
E	P85	P86	P87	P88	C45	C46	C47	C48	C49	C50	C51	C52
F	C53	C54	C55	C56	C57	C58	C59	C60	C61	C62	C63	C64
G	C65	C66	C67	C68	C69	C70	C71	C72	C73	C74	C75	C76
H	C77	C78	C79	C80	C81	C82	C83	C84	C85	C86	C87	C88

Figure 14: Plate 2 Design

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std.1	Std.2	Std.3	Std.4	Std.5	Std.6	Std.7	B	P89	P90	P91	P92
B	P93	P94	P95	P96	P97	P98	P99	P100	P101	P102	P103	P104
C	P105	P106	P107	P108	P109	P110	P111	P112	P113	P114	P115	P116
D	P117	P118	P119	P120	P121	P122	P123	P124	P125	P126	P127	P128
E	P129	P130	P131	P132	C89	C90	C91	C92	C93	C94	C95	C96
F	C97	C98	C99	C100	C101	C102	C103	C104	C105	C106	C107	C108
G	C109	C110	C111	C112	C113	C114	C115	C116	C117	C118	C119	C120
H	C121	C122	C123	C124	C125	C126	C127	C128	C129	C130	C131	C132

Figure 15: Plate 3 Design

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std.1	Std.2	Std.3	Std.4	Std.5	Std.6	Std.7	B	P133	P134	P135	P136
B	P137	P138	P139	P140	P141	P142	P143	P144	P145	P146	P147	P148
C	P149	P150	P151	P152	P153	P154	P155	P156	P157	P158	P159	P160
D	P161	P162	P163	P164	P165	P166	P167	P168	P169	P170	P171	P172
E	P173	P174	P175	P176	C133	C134	C135	C136	C137	C138	C139	C140
F	C141	C142	C143	C144	C145	C146	C147	C148	C149	C150	C151	C152
G	C153	C154	C155	C156	C157	C158	C159	C160	C161	C162	C163	C164
H	C165	C166	C167	C168	C169	C170	C171	C172	C173	C174	C175	C176

Figure 16: Plate 4 Design

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std.1	Std.2	Std.3	Std.4	Std.5	Std.6	Std.7	B	P177	P178	P179	P180
B	P181	P182	P183	P184	P185	P186	P187	P188	P189	P190	P191	P192
C	P193	P194	P195	P196	P197	P198	P199	P200	P201	P202	P203	P204
D	P205	P206	P207	P208	P209	P210	P211	P212	P213	P214	P215	P216
E	P217	P218	P219	P220	C177	C178	C179	C180	C181	C182	C183	C184
F	C185	C186	C187	C188	C189	C190	C191	C192	C193	C194	C195	C196
G	C197	C198	C199	C200	C201	C202	C203	C204	C205	C206	C207	C208
H												

Figure 17: Plate 5 Design

2.5 Statistical Analysis

For the statistical analysis of the data, Microsoft Office excel and SPSS (Statistical Package for Social Sciences) software were used. A Pearson Chi-Square test was used to determine the significance of sociodemographic properties between OCD patients and healthy controls. To distinguish between patient and control group an independent samples t-test was done. Descriptive statistics were used to analyze the sociodemographic characteristics of the study participants and the results were presented as mean ± SEM. A p value below 0.05 was regarded as statistically significant.

Chapter: 3

3.1 Results

3.1.1 Socio-demographic Profiles

Table 3: Socio-demographic profiles of study participants

Characteristics	Patients (n=220)		Controls (n=208)		p-value
	n	%	n	%	
Marital Status					0.369
Married	91	41.4	95	45.6	
Unmarried	129	58.6	113	54.4	
Education Level					0.002
Illiterate	5	2.3	8	3.9	
Primary	9	4.1	29	13.9	
Secondary	123	55.9	94	45.2	
Graduate and above	83	37.7	77	37	
Occupation					<0.001
Business	6	2.7	0	0	
Service	50	22.7	77	37	
Housewife	22	10	50	24	
Student	66	30	0	0	

Unemployed	75	34.1	80	38.5	
Other	1	0.5	1	0.5	
Residence					<0.001
Rural	38	17.3	81	38.9	
Urban	182	82.7	127	61.1	
Economic Impression					<0.001
High	17	7.7	0	0	
Low	79	35.9	64	30.8	
Medium	124	56.4	144	69.2	
Smoking History					0.005
Non-smoker	193	87.7	161	77.4	
Smoker	27	12.3	47	22.6	

Table 3 shows the socio-demographic status of the study participants. In this study, 220 patients who were suffering from OCD and 208 healthy controls were included.

Among patients, 41.4% were married and 58.6% were unmarried compared to controls where 45.6% were married and 54.4% were unmarried. The difference in two groups was not statistically significant (p=0.369). In the case of education level, 2.3% patients and 3.9% controls were illiterate. Primary education was observed in 4.1% patients and 13.9% controls. Secondary education was reported by 55.9% patients and 45.2% controls. Graduate and above level education was found in 37.7% patients and 37% controls. The difference between two groups was statistically significant (p=0.002).

Among patients, 2.7% were engaged in business, 22.7% in service, 10% were housewives, 30% were students, 34.1% were unemployed and 0.5% belonged to other occupations. In the control group, none were involved in business or student categories while 37% were in service, 24% were housewives, 38.5% were unemployed and 0.5% belonged to other occupations. The difference in occupation distribution was statistically significant ($p<0.001$).

In terms of residence, 17.3% patients lived in rural areas and 82.7% lived in urban areas. Among the controls, 38.9% were from rural areas and 61.1% were from urban areas. The difference between two groups was statistically significant ($p<0.001$). Among the patients, 7.7% belonged to the high economic group, 35.9% to the low economic group and 56.4% to the medium economic group. In the control group, none were in the high economic group while 30.8% were in the low economic group and 69.2% were in the medium economic group. The difference in economic distribution between two groups was statistically significant ($p<0.001$).

Regarding smoking history, 87.7% patients were nonsmokers and 12.3% were smokers. In the control group, 77.4% were nonsmokers and 22.6% were smokers. The difference in smoking status between patients and control was statistically significant ($p=0.005$).

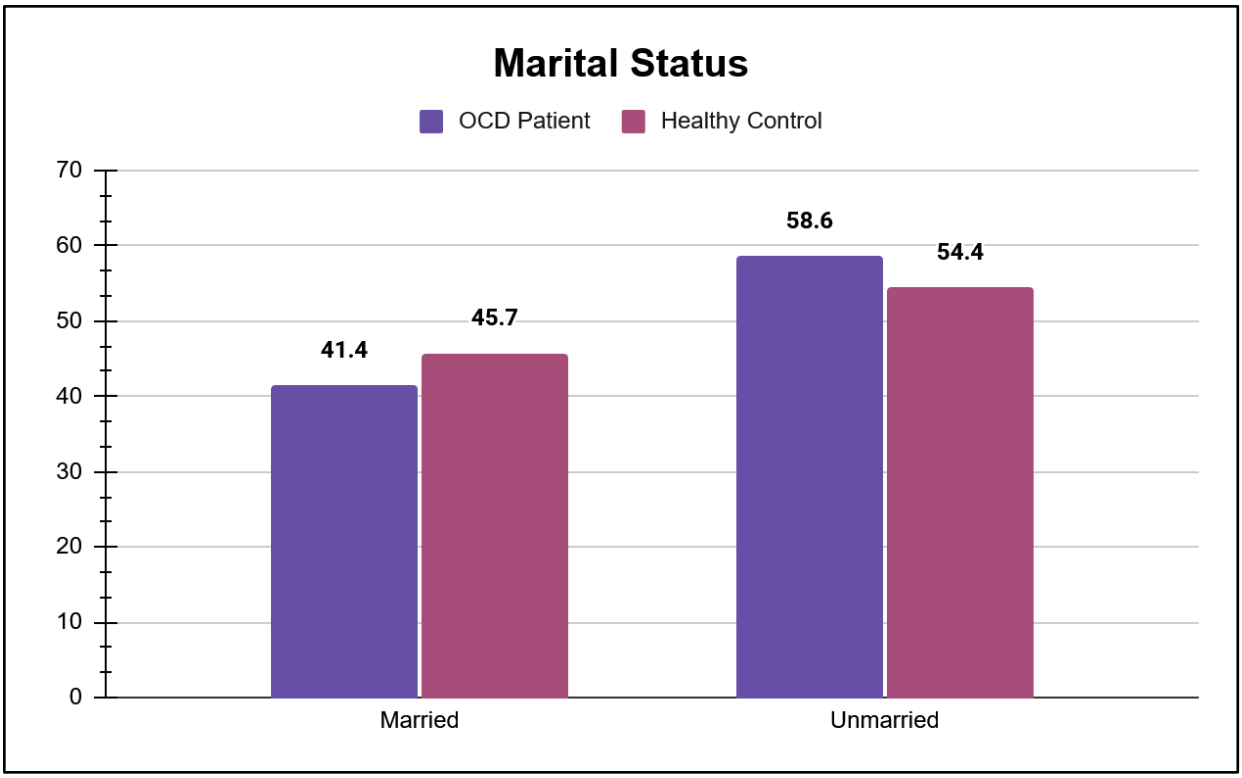

Figure 18: Distribution of marital status among OCD patients and healthy control

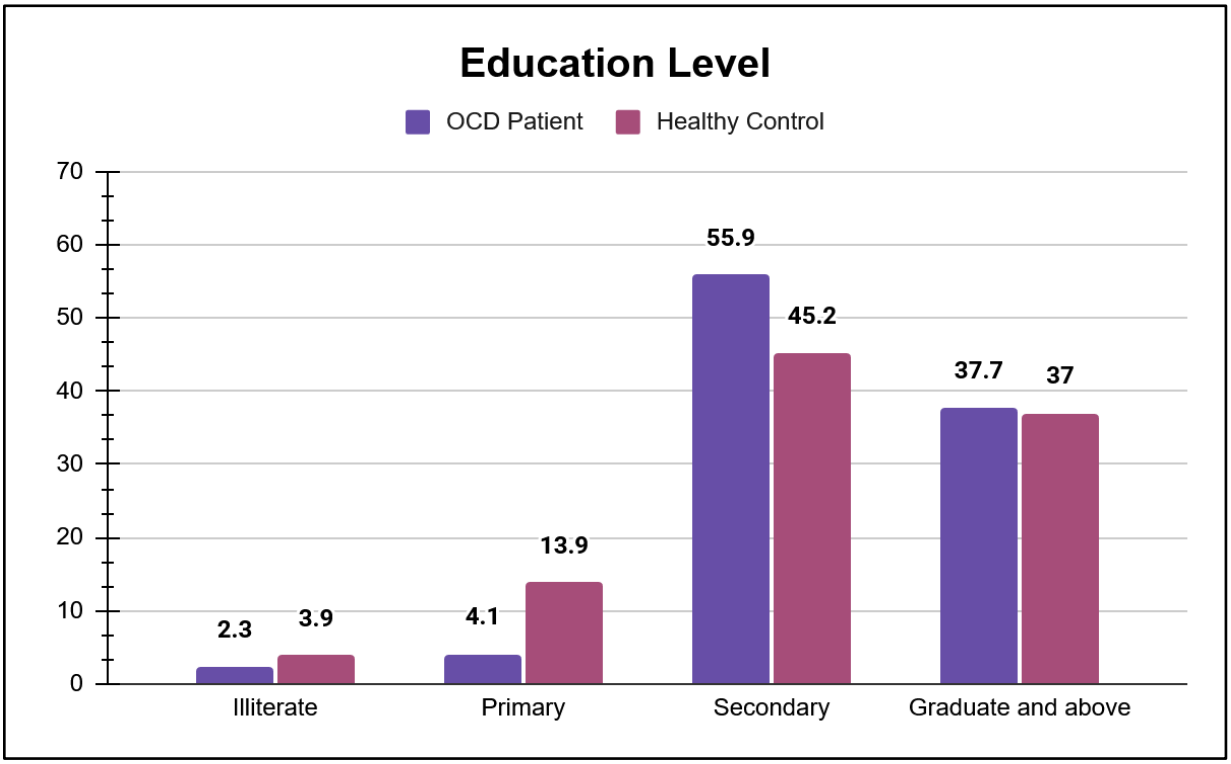

Figure 19: Distribution of Education level among OCD patients and healthy control

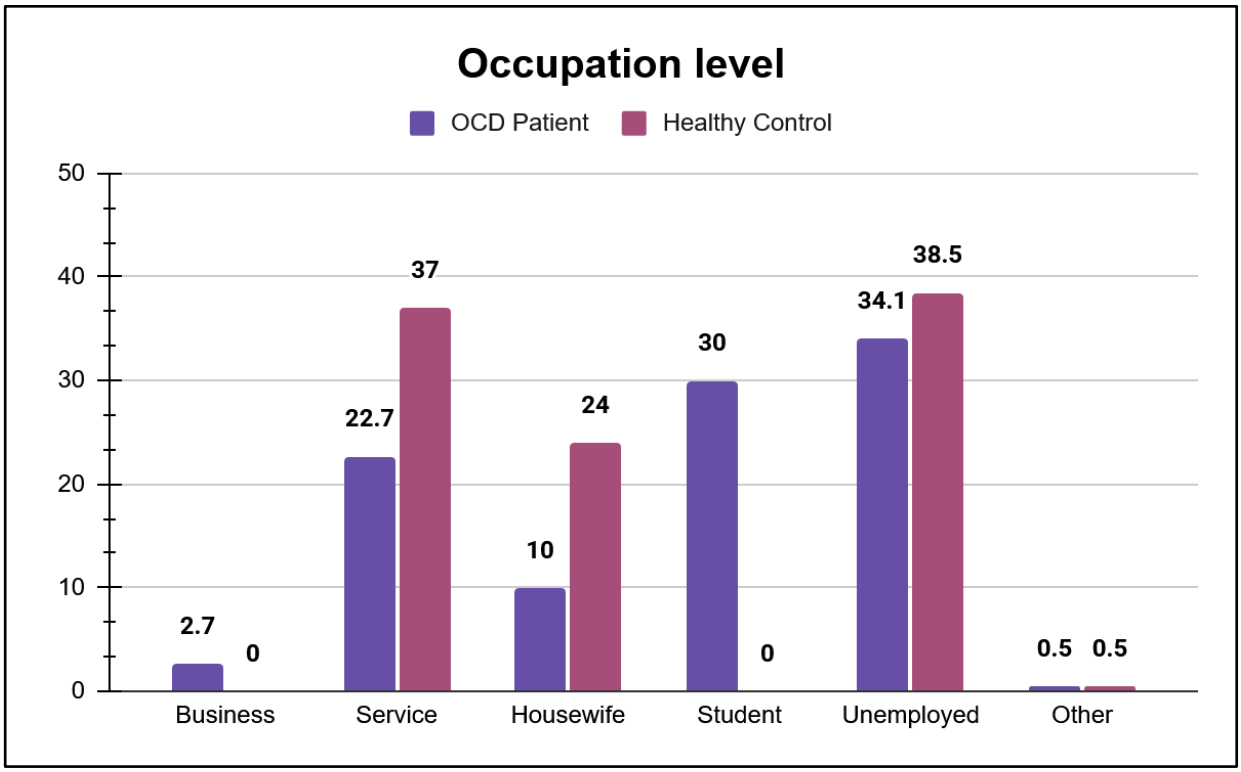

Figure 20: Distribution of Occupation level among OCD patients and healthy control

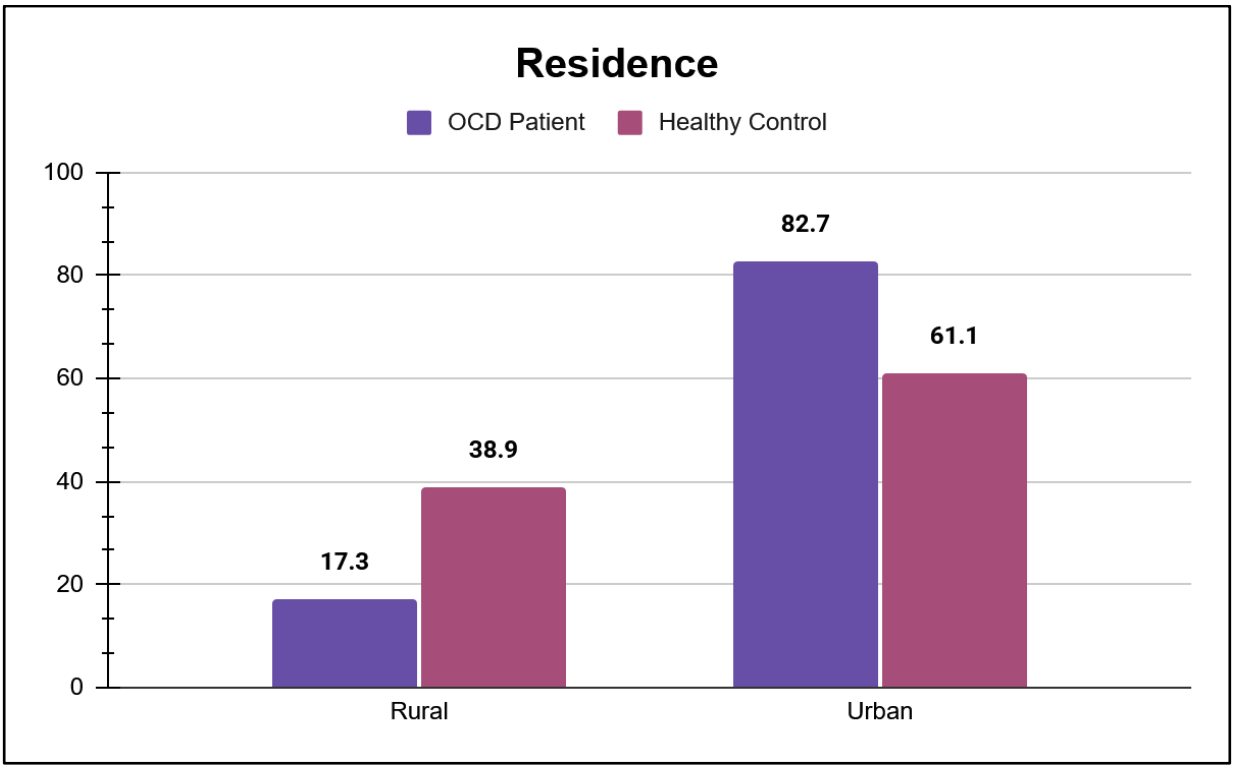

Figure 21: Distribution of Residence among OCD patients and healthy control

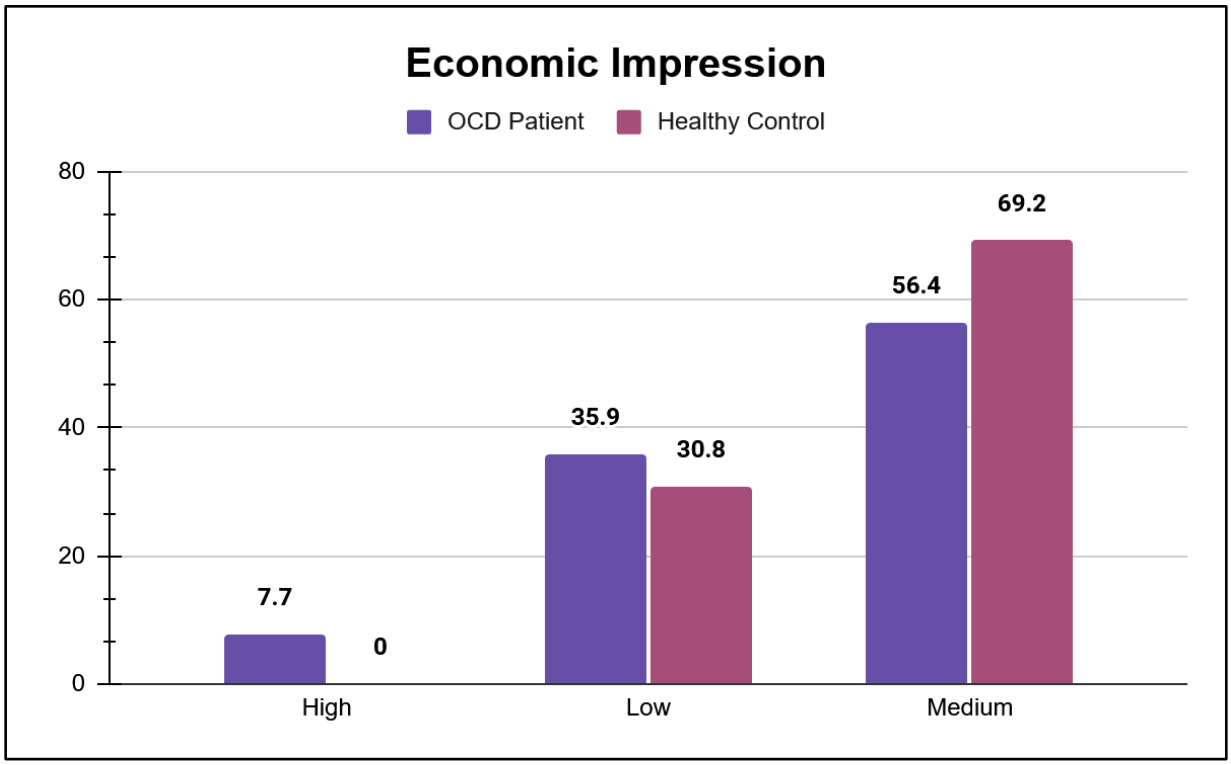

Figure 22: Distribution of Economic Impression among OCD patients and healthy control

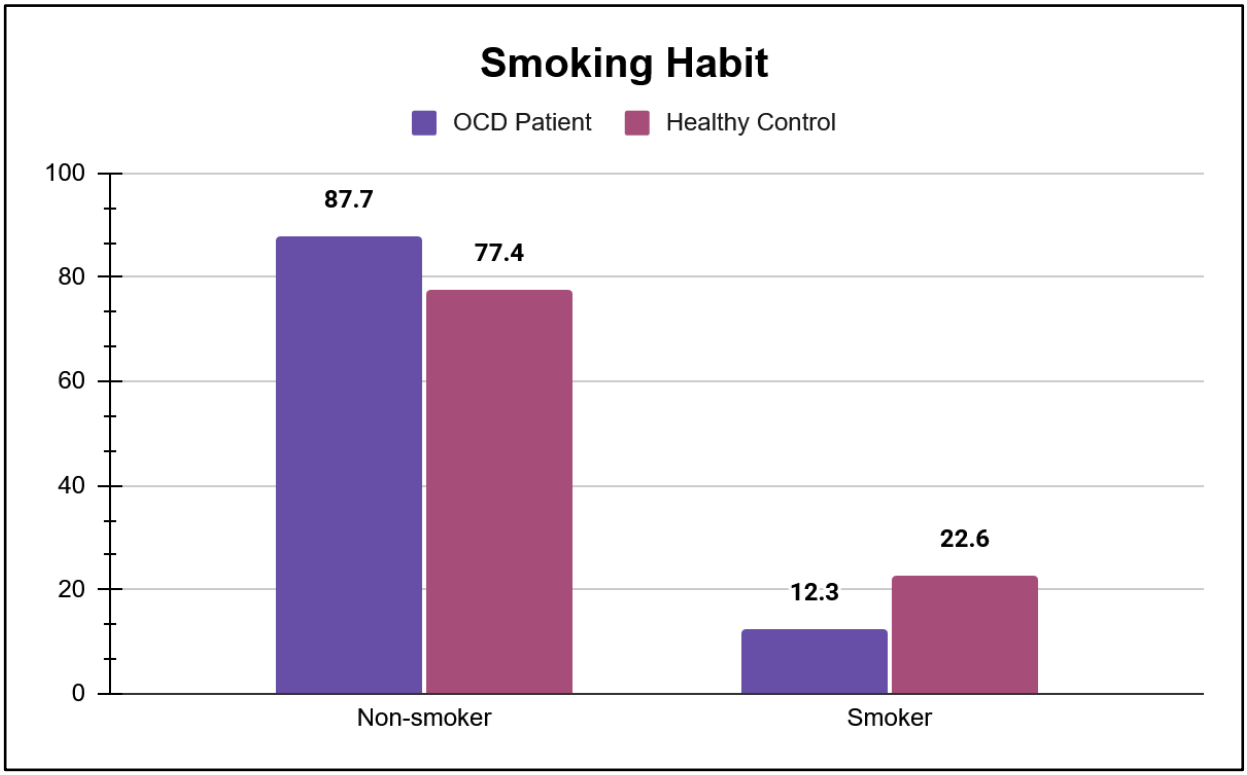

Figure 23: Distribution of Smoking Habit among OCD patients and healthy control

3.1.2 Biophysical Profiles

Table 4: Biophysical profiles of study participants

Characteristics	Patients (n=220)		Control (n=208)		p-value
	n	%	n	%	
Age in years					0.639
18-25	126	57.3	111	53.4	
26-35	53	24.1	56	26.9	
36-45	32	14.5	28	13.5	
46-60	9	4.1	13	6.2	
Gender					0.220
Female	105	47.7	87	41.8	
Male	115	52.3	121	58.2	
BMI (kg/m^2)					0.095
CED (below 18.5)	16	7.3	18	8.6	
Normal (18.5-25.0)	149	67.7	120	57.7	
Overweight (above 25.0)	55	25	70	33.7	

Table 4 shows the biophysical profiles of the study participants. The age distribution showed that 57.3% patients and 53.4% controls were aged between 18 to 25 years. Participants aged between 26 to 35 years included 24.1% patients and 26.9% controls. In the 36-45 years group, there were

14.5% patients and 13.5% controls. Participants aged between 46 to 60 years included 4.1% patients and 6.2% controls. The difference in age distribution was not statistically significant (p=0.639).

Among patients, 47.7% were female and 52.3% were male. In the control group, 41.8% were female and 58.2% were male. The difference in gender distribution was not statistically significant (p=0.220). In case of BMI, 7.3% patients and 8.6% controls were in the chronic energy deficiency (CED) group (BMI <18.5). Normal BMI (18.5-25.0) was observed in 67.7% patients and 57.7% controls. Overweight (BMI >25.0) was found in 25% patients and 33.7% controls. The difference in BMI distribution was not statistically significant (p=0.095).

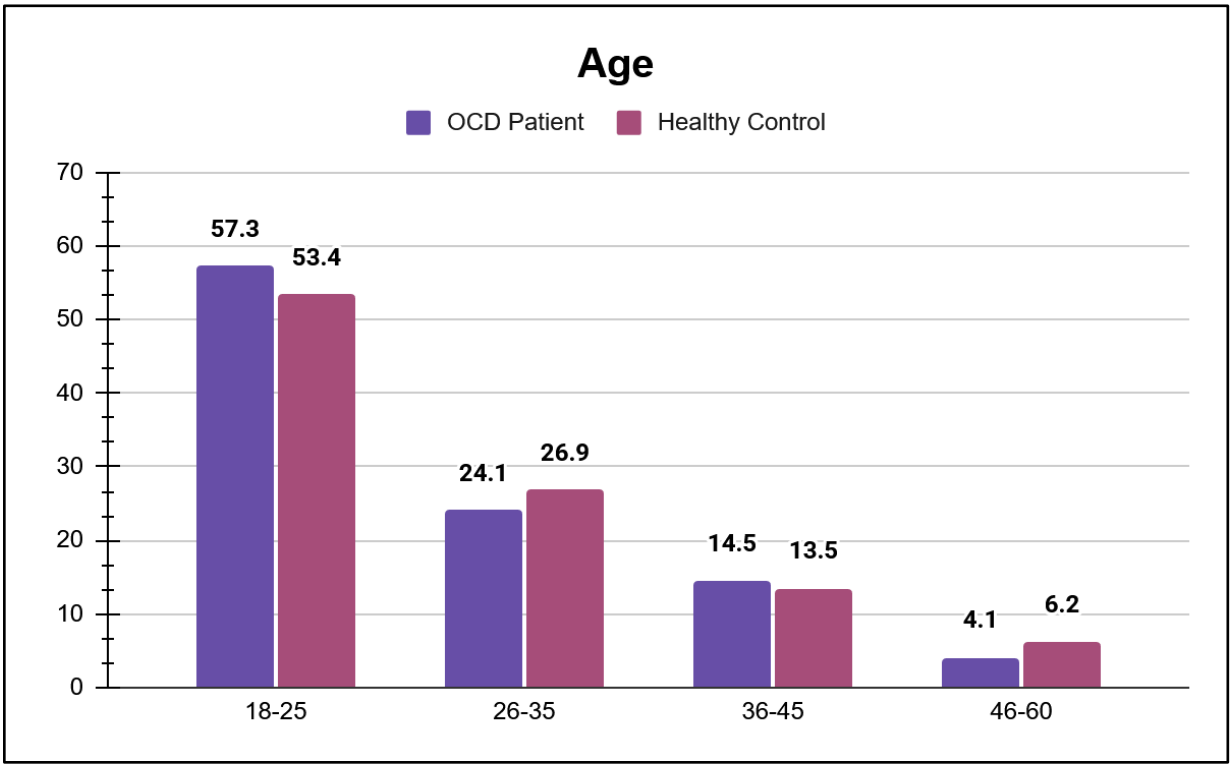

Figure 24: Distribution of Age among OCD patients and healthy control

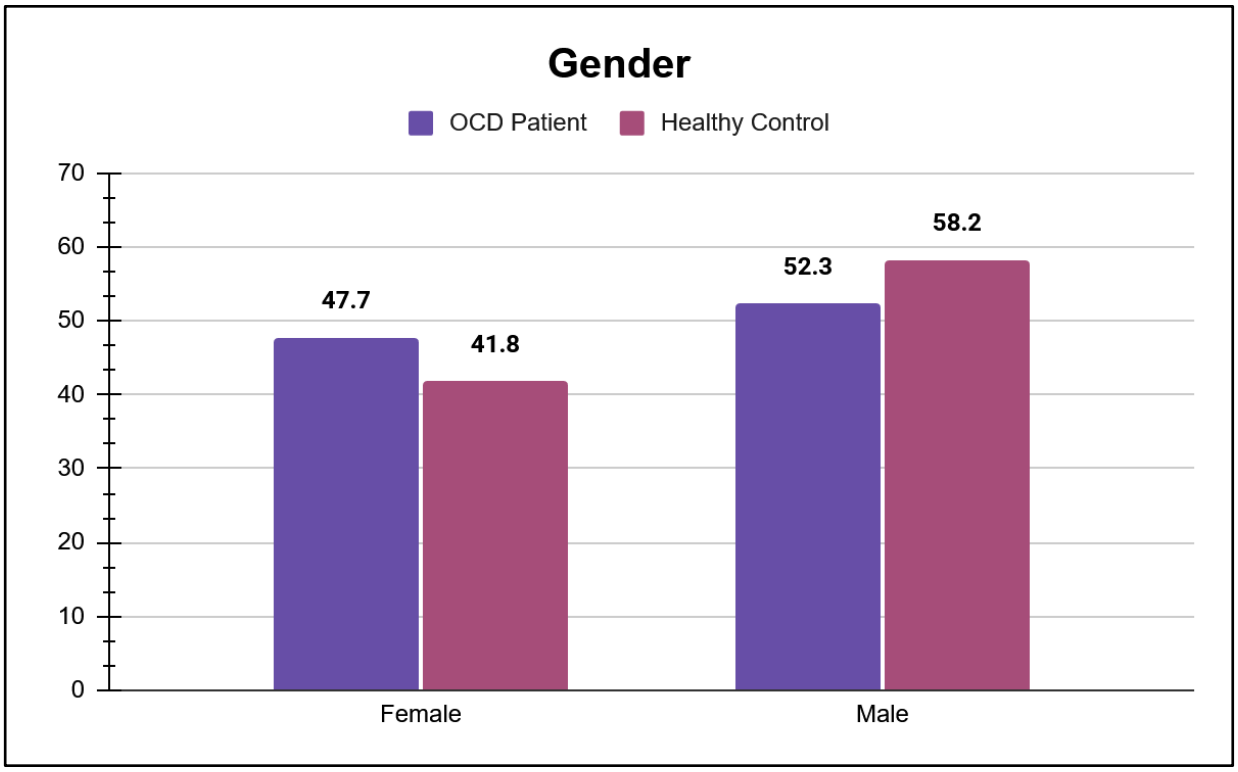

Figure 25: Distribution of Gender among OCD patients and healthy control

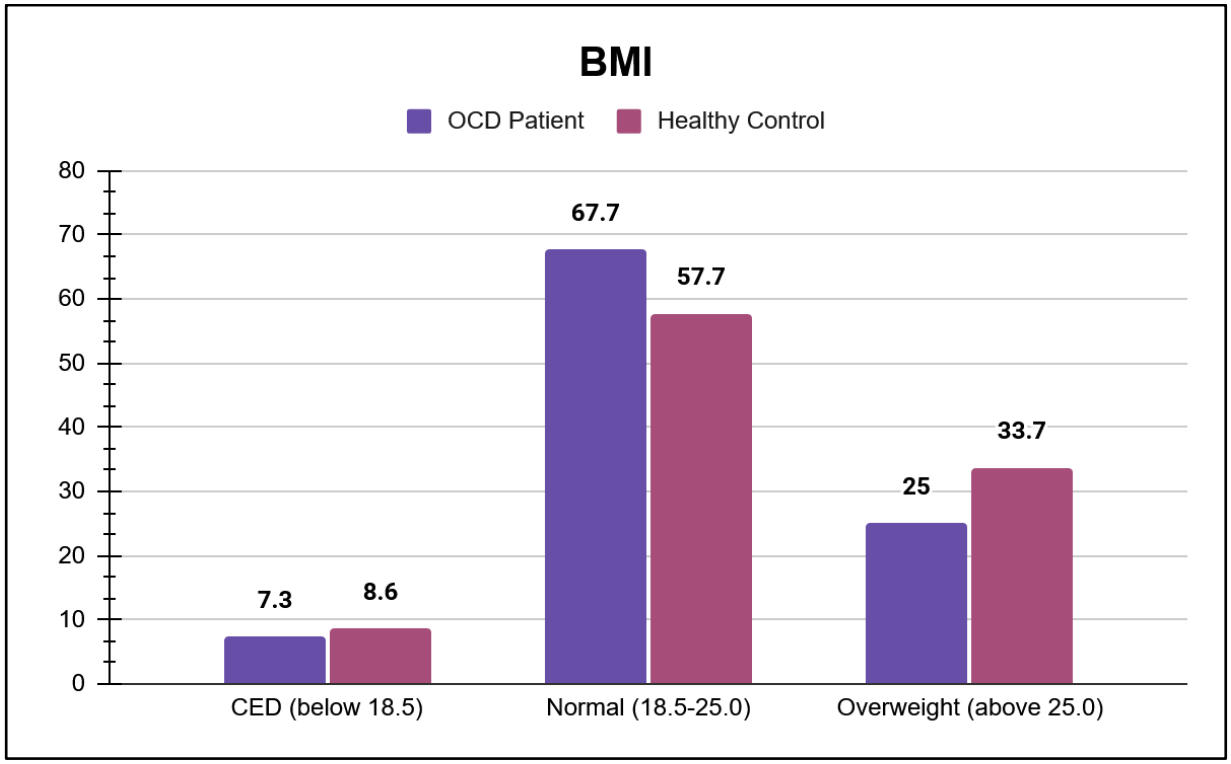

Figure 26: Distribution of BMI among OCD patients and healthy control

3.1.3 Clinical Profile and Laboratory Findings

Table 5: Clinical profile and laboratory findings of study participants

Parameters	Groups	n	Mean ± SEM	p-value
Age in years				0.477
	Patients	220	27.9 ± 0.551	
	Controls	208	28.5 ± 0.643	
BMI (kg/m^2)				0.906
	Patients	220	23.4 ± 0.256	
	Controls	208	23.4 ± 0.27	
Y-BOCS				<0.001
	Patients	220	20.62 ± 0.497	
	Controls	208	3.45 ± 0.123	
Concentration of MIF (ng/ml)				0.001
	Patients	220	6.73 ± 0.183	
	Controls	208	5.14 ± 0.286	

Table 3.3 shows the clinical profile and laboratory findings of the study participants. The mean age of patients was 27.9 ± 0.551 years while the mean age of controls was 28.5 ± 0.643 years. The difference in mean age between the two groups was not statistically significant (p=0.477). The mean BMI of patients was 23.4 ± 0.256 kg/m^2 whereas the mean BMI of controls was also 23.4 ±

0.27 kg/m^2. There was no statistically significant difference between the BMI of the groups (p=0.906).

The mean Y-BOCS score among patients was 20.62 ± 0.497 and in controls it was 3.45 ± 0.123. The difference in mean Y-BOCS scores between the groups was statistically significant (p<0.001). The mean concentration of MIF among patients was 4.91 ± 0.183 ng/ml and in controls it was 6.05 ± 0.286 ng/ml. The difference between patient and control was statistically significant (p=0.001).

3.2 Standard Curve for MIF

Table shows the concentration of the MIF standards and absorbances that were used to prepare the standard curve.

Table 6: MIF standard concentration and absorbance

Concentration (ng/mL)	Absorbance
1000	1.2795
500	0.5808
250	0.1718
125	0.0525
63	0.048
31	0.0443
16	0.0377

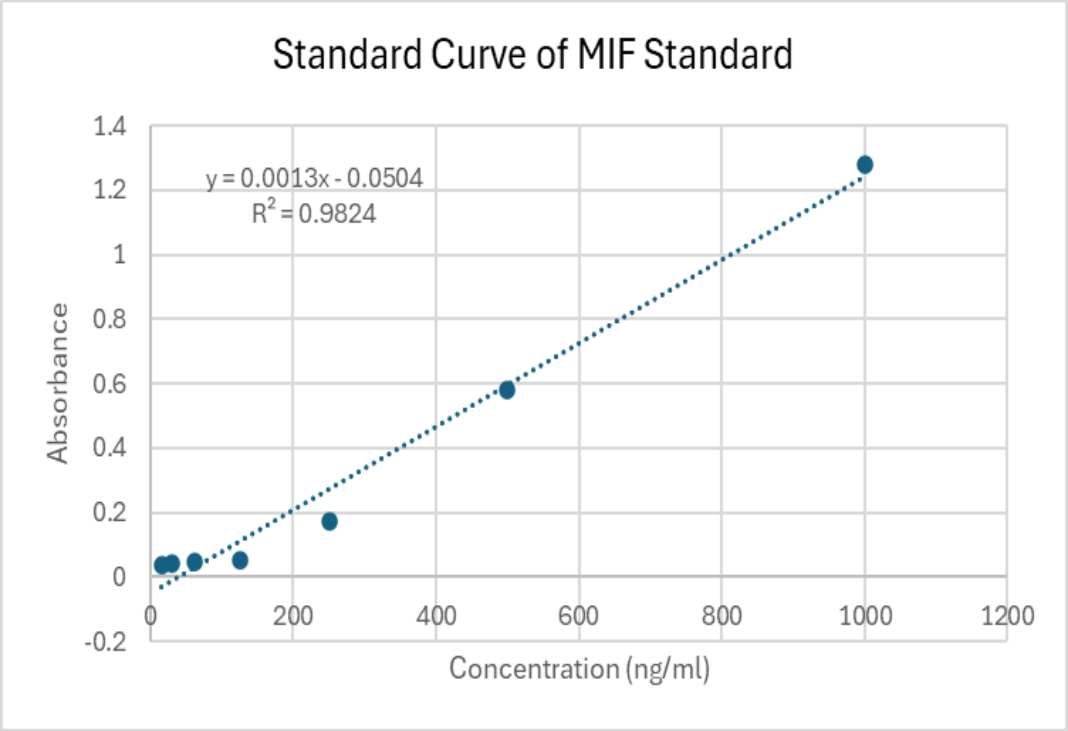

Figure 27: Standard Curve of MIF Standard

Chapter 4

Discussion

Various research has been done on the biological markers of OCD to determine a relation between these indicators and OCD. But none of the studies on the biomarkers has been able to demonstrate enough sensitivity and specificity and thus none are used for diagnostic studies till now (Fullana et al., 2020). To help contribute in this field, this study was designed to find out the relationship of MIF in OCD. This case control study was conducted to evaluate the serum levels of MIF in OCD patients and healthy controls. The serum MIF levels were significantly increased in patients with OCD (6.73 ± 0.183 ng/ml) compared with healthy controls (5.14 ± 0.286 ng/ml).

MIF plays a central role in regulating inflammatory responses and modulating glucocorticoid activity which indicates that there is a potential link between immune activation and stress related psychiatric disorders like OCD (Sumaiya et al., 2022). Higher levels of MIF have been related with depressive symptoms and a weaker cortisol response to stress (Edwards et al., 2010). MIF signaling influences how the body responds to stress through the neuroendocrine system. It may disrupt normal cortisol regulation and can lead to an abnormal stress response. This disruption could be a possible pathway that increases the risk of stress related mental health disorders like OCD (Lipschutz et al., 2018). MIF may be linked to OCD through evidence found in autism. Studies show that higher levels of MIF in the plasma are associated with greater severity of symptoms in autism spectrum disorder (ASD) (Grigorenko et al., 2008). Since both autism and OCD involve compulsive and repetitive behaviors, it might suggest that MIF can also be related in OCD. Therefore, MIF may play a role influencing the brain circuits that are responsible for compulsive behaviors.

Although no previous studies have specifically evaluated MIF levels in OCD patients, the present findings of this study are consistent with previous studies that shows higher level of pro inflammatory cytokines like IL-6 and TNF-α in people with OCD which suggest that inflammation may be involved in the disorder (Rodríguez et al., 2017). The higher MIF levels found in this study also match earlier reports that show increased MIF in depression and stress related conditions where it is linked to immune activation and changes in HPA axis function (Bay-Richter et al., 2015).

Although several studies suggest that immune system problems and inflammation may play a role in OCD, there is very little research on MIF. Based on available information, this is among the first studies to examine MIF levels in OCD patients and offers new evidence that suggests that there might be some links to this cytokine to the disorder. By studying MIF levels, this research investigates a less studied inflammatory factor in OCD and might help better understand the disorder. This study will provide data from the Asian region to the global research and might help improve understanding of OCD related immune changes across different populations and cultures.

The findings of this study may have several important uses in clinical practice. They can help improve how we understand, diagnose and treat OCD from a biological point of view. The elevated MIF levels in OCD patients suggest that inflammation may be involved in the disorder. This supports the use of biological markers along with traditional clinical assessments and might help create a more complete understanding of OCD. This research findings may contribute and raise the possibility of exploring anti-inflammatory or immune targeting treatments in OCD. In the future, MIF might be used as part of a group of biomarkers to help assess OCD more objectively.

This short time study limits the ability to make cause and effect conclusions. The elevated MIF levels in OCD patients compared with healthy controls might suggest a link but it is not clear whether these changes cause OCD or result from it. Long term studies are needed to understand how immune changes and OCD symptoms develop over time. The sample size may also limit how widely the finding can be applied. Larger studies from multiple centers are needed to better represent people with OCD. Some factors that can affect the immune system may not have been fully controlled. Factors like recent mild infection, sleep cycle, diet and other medical conditions can change MIF levels and may have influenced the results. Future studies should better control factors that may affect the result such as medication use, other mental or physical illness, lifestyle habits and inflammation levels.

Chapter 5

Conclusion

This study investigated serum MIF levels in patients with OCD compared to healthy controls. The findings of this study provide preliminary evidence of a possible association between serum MIF levels and OCD which may support the possible role of immune and inflammatory mechanisms in the pathophysiology of OCD. In the clinical research area, MIF may be open for discussion as a useful supporting biomarker to help understanding disease mechanisms, monitoring illness activity or guiding future immune based additional treatments. However, it is not ready for routine clinical use yet. Future studies should include larger, long term and multi center designs to confirm the reliability, specificity and sensitivity of the result, control for other influencing factors and examine how MIF is related to symptom severity and treatment response.

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