Evaluation of Serum Chemokine (C-X-C Motif) Ligand 4 Levels among Obsessive Compulsive Disorder Patients in Bangladesh

By

Salvina Chowdhury Neha

22346020

A thesis submitted to the School of Pharmacy for the Partial

fulfillment of requirements for the degree of

Bachelor of Pharmacy

School of Pharmacy

Brac University

May 2026

Declaration

It is hereby declared that

1. The thesis submitted is my 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 have acknowledged all main sources of help.

Student's Full Name & Signature:

Salvina Chowdhury Neha
22346020

Approval

The project titled "Evaluation of Serum Chemokine (C-X-C motif) Ligand 4 Levels among Obsessive Compulsive Disorder Patients in Bangladesh" submitted by Salvina Chowdhury Neha of spring, 2026 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on 7th May 2026.

Examining Committee:

Supervisor:

_____

Dr. Md. Rabiul Islam
Associate Professor, School of Pharmacy
Brac University

Program Coordinator:

_____

Namara Mariam Chowdhury
Senior Lecturer and Program Coordinator
School of Pharmacy
Brac University

Chairperson:

_____

Dr. Sharmind Neelotpol, PhD
Professor, School of Pharmacy
Chairperson, Department of Pharmacy
Brac University

Ethics Statement

The study was conducted in compliance with the principles of Helsinki Declaration and Bangladesh Medical Research Council (BMRC) guidelines. Before initiation of this project, the ethical clearance was obtained from Southeast University of Bangladesh. A consent form with the participants signature was taken from all participants before enrolling them after giving a brief overview of the purpose, objectives, benefits, the process and possible risks of the study. During sample collection, participant safety and comfort were ensured. A qualified healthcare professional was responsible for withdrawing blood samples from the participants in an aseptic manner. Throughout the project, confidentiality of all participants was maintained, and the participants had the choice to withdraw at any time. All local and international ethical standards were taken into consideration to maximize the safety of the participants.

Abstract

The study aimed to evaluate the serum levels of pro-inflammatory chemokine (C-X-C motif) ligand 4 (CXCL4) between obsessive compulsive disorder (OCD) patients and healthy individuals. The study was conducted with a total of 376 participants, who were evaluated based on multiple biophysical, sociodemographic and clinical parameters. No variance was detected in all the parameters except BMI. The BMI was found to be statistically significant, but it lacked clinical relevance. Yale-Brown Obsessive Compulsive Scale (Y-BOCS) score demonstrated consistent and significant severity of OCD in between the two groups. Statistical analysis indicated a reduction in serum CXCL4 levels in the patients with respect to the serum level of the control, the findings were not statistically significant. Any possible involvement of CXCL4 in disease pathology could not be detected, CXCL4 demonstrates poor credibility as a biomarker in diagnosis of OCD. This study having good generalizability and dependability, had few limitations, including the single biomarker design.

Keywords: *Obsessive Compulsive Disorder, Cytokine, CXCL4, biomarkers, pathophysiology, neuroinflammation.*

Dedication

I dedicate this project to my parents for their unwavering support throughout this journey.

Acknowledgement

I would like to express my deepest gratitude to my parents for always supporting and encouraging me, which built the foundation of what I can achieve. I am grateful to my family for all the sacrifices, care and love that I received. And, to my feathered friends whose silent presence was the loudest source of comfort. My sincerest gratitude to Dr. Md. Rabiul Islam, my supervisor, for guiding me and allowing me to be a part of such a huge project. I will always be grateful for this opportunity to the school of pharmacy and my supervisor, whose guidance introduced me to and sparked new interests in me regarding such research projects. I hope to carry on this moment and energy with me to the future.

Table of Contents

List of Tables

Table no. **Page no.**

List of Figures

Figure no. **Page no.**

List of Acronyms

Acronym	Full Form
OCD	Obsessive Compulsive Disorder
CXCL4	Chemokine (C-X-C Motif) Ligand 4
DSM	Diagnostic and Statistical Manual of Mental Disorders
ICD	International Classification of Diseases
OCRD	Obsessive Compulsive and Related Disorder
CSTC	Cortico-Striato-Thalamo-Cortical
Y-BOCS	Yale-Brown Obsessive Compulsive Scale
SSRI	Selective Serotonin Reuptake Inhibitor
CBT	Cognitive Behavioral Therapy
PFC	Prefrontal Cortex
ACC	Anterior Cingulate Cortex
dlPFC	Dorsolateral Prefrontal Cortex
vmPFC	Ventromedial Prefrontal Cortex
OFC	Orbitofrontal Cortex
GPe	Globus Pallidus Externa
NAc	Nucleus Accumbens

STN	Subthalamic Nucleus
SNRIs	Serotonin-Norepinephrine Reuptake Inhibitors
ERP	Exposure and Response Prevention
PF4	Platelet Factor 4
GAG	Glycosaminoglycan
bFGF	Basic Fibroblast Growth Factor
VEGF	Vascular Endothelial Growth Factor
HSPGs	Heparan Sulfate Proteoglycans
IL-8	Interleukin-8
ELISA	Enzyme Linked Immunosorbent Assay
HCs	Healthy Controls
BMI	Body Mass Index
SPSS	Statistical Package for Social Sciences
SEM	Standard Error of the Mean

Chapter 1

Introduction

1.1 Background of Obsessive-compulsive disorder

1.1.1 Definition

Obsessive compulsive disorder (OCD) is a prevalent disabling neuropsychiatric condition and usually a chronic disorder (Goodman et al., 2014). It is manifested by intrusive and undesired thoughts or recurrent urges that are labelled as obsession, followed by compulsions which are recurrent behaviours that an individual exhibits for the means of neutralizing the obsession (American Psychiatric Association, 2013; Goodman et al., 2014; Barlow, 2014).

1.1.2 Obsession- Compulsion

According to the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5), obsession can be defined as frequently encountered intrusive, unwanted and persistent urges or thoughts that become a nuisance for an individual, commonly associated with noticeable anxiety and distress (American Psychiatric Association, 2013). Some commonly observed acts of obsession include- constantly desiring for symmetry, constant feeling of incompleteness, inappropriate sexual thoughts, recurrent unwanted aggressive actions towards people, concerns about harm, sacrilegious thoughts and as well as fear of contamination such as germs, dirt, body waste or illness etc. (Storch et al., 2010). Whereas, compulsions are activities that an individual feels compelled to perform for neutralizing the urges of obsession or it can be defined as mental or behavioural action intended for alleviating anxiety or distress caused by obsessions; where these acts are disproportionately extreme, not realistically aligning with what they want to neutralize(American Psychiatric Association, 2013). Compulsions are mostly noticeable acts, but they can also be mental rituals that go unnoticed. Hoarding, over sanitizing, unnecessary cleaning, repeatedly rearranging objects, etc. are some common types of noticeable acts of compulsion. On the other hand, consistently mentally reaffirming all tasks were completed, constant recitation of prayers for preventing appalling thoughts are some examples of unobservable mental rituals (Storch et al., 2010).

1.1.3 Functional consequences

A correlation between OCD and reduced quality of life have been observed throughout the years of studying this disorder. Functional consequences such as social and occupational

difficulties were also found to be linked to OCD, which results from the time lost by obsessing and conducting compulsive activities (American Psychiatric Association, 2013). A study by Hollander et al. (1998) conducted on individuals with OCD states that the percentage of OCD induced family problems, impaired socialization, academic difficulties and occupational difficulties in the test population were documented to be 70%, 63%, 59% and 60% respectively. The intensity of these functional consequences depends on the severity of the symptom. Different symptoms of OCD can lead to variation in function restriction. For instance, people concerned with contamination and fear of germs might avoid going to the hospital and doctors, leading to various health concerns including dermatologic issues. Again, individuals having an obsession with harm may distance oneself from their family members and loved ones as a precaution to avoid any hazardous situation. In some cases, the symptoms are so prevalent that it hinders treatment. For instance, avoiding taking medications considering them to be contaminated (American Psychiatric Association, 2013).

Moreover, manifestations of OCD in children and adolescents have been linked with developmental difficulties (American Psychiatric Association, 2013). A study by Piacentini et al. (2003) reveals that 90% of the total young population assessed had OCD induced functioning difficulties. Another study highlighting the link between OCD and its effect on psychology concludes that individuals with OCD tend to have lower ability in both theory of mind and facial emotion recognition, compared to the control participants. These traits of individuals with OCD along with their perceived lack of cognitive empathy leads to social and interpersonal difficulties (Bora, 2022).

1.1.4 Classification

Previously OCD was interpreted as an anxiety disorder. However, considering a key factor that is the heterogeneity of emotional experiences associated with obsession, OCD can be differentiated from Anxiety and Fear-Related Disorders (Stein et al., 2016). Thus, the DSM-5 5th edition and the World Health Organization's International Classification of Diseases and Related Health Problems (ICD-11) has grouped it into a distinctive OCD and related disorders (OCRDs) highlighting unique neurological and clinical differences. The DSM-5 5th edition grouping includes body dysmorphic disorder, excoriation or skin-picking disorder, hoarding disorder, trichotillomania alongside OCD within OCRDs (American Psychiatric Association, 2013). Concurrently, the ICD-11 classification encompasses hypochondriasis, olfactory reference disorder along with OCD, body dysmorphic disorder and hoarding disorder emphasizing an increased degree of similarity among these conditions. This basis of

classification has been validated by numerous data from general and neurochemical studies demonstrating similar clinical features i.e. repetitive behaviours. Some evidenced similarities include genetic risk factors, neurochemical abnormalities, and as well as underlying neurocircuitry anomalies (Stein et al., 2016).

A brief insight into the related disorders is as follows-

1. Body dysmorphic disorder

This is a mental condition where an individual has preoccupation with perceived flaws in one's own physical appearance. These flaws are self-perceived and not visible to others and are considered extreme. It is characterized by repetitive behaviours such as checking on one's facial features, skin pecking, seeking validation regarding appearances, comparison of appearance with others etc. (American Psychiatric Association, 2013). Body dysmorphic disorder, like OCD, causes intrusive thoughts, repetitive behaviour and has high rates for cooccurrence with OCD. Hence, it was included within the class of OCRD (Stein et al., 2016).

2. Hypochondriasis

Hypochondriasis is another disorder which deals with consistent presence of intrusive thoughts and repetitive actions. Thus, it was included among the OCRDs. This condition deals with an individual's preoccupation with the probability of suffering from illnesses. Frequently requiring assistance and reassurance from healthcare professionals, repeated screening for possible symptoms of serious diseases and as well as seeking disease related information are some commonly observed repetitive behaviors (Stein, 2012; Rachman, 2012).

3. Hoarding disorder

In case of hoarding disorder, repetitive behaviour can be observed in case of excessive acquisition of goods, attachment towards these items and constant hesitation in discarding these goods (Stein et al., 2016). It should be noted that collection is different from hoarding. In hoarding disorder, individuals refuse to discard objects, often regardless of these having very low value. In the long term this disorder results in possessions being piled up in the living areas thereby causing disorganization, congestion and compromises their use (American Psychiatric Association, 2013). Phenomenological similarities of hoarding disorder with OCD, which is repetitive behaviour observed to avoid distress, is the basis of classifying hoarding disorder among the OCRDs (Stein et al., 2016).

4. Olfactory reference disorder

Olfactory reference disorder is a mental condition which is characterized by a constant false perception of producing bad odour. The individual usually constantly tries to detect the source of smell followed by attempts to mask the odour. Individuals usually suffer from anxiety, shame and avoid social gatherings. This disorder is commonly comorbid with OCD (Stein et al., 2016).

1.2 Epidemiology of Obsessive-Compulsive Disorder

1.2.1 Global Prevalence

Among the global population the occurrence of OCD is sufficiently high, currently placing 4th among the most prevalent mental disorders (Kessler et al., 2005). Earlier estimates suggested that the percentage of OCD patients among the general population is 0.05%, making it a rare mental condition (Cervin, 2023). At present, globally, OCD has been reported to affect approximately 2-3% of the entire population (Goodman et al., 2014). According to a recent meta-analysis and systematic review, the likelihood of lifetime prevalence of OCD in adults was reported to be 1.3% (Cervin, 2023). Such rates were also evident in children and adolescents. For instance, in the US the lifetime and current prevalence of OCD among adolescents (14-19 year olds) is 1.9% and 1% respectively (Cervin, 2023). In Europe, OCD has an annual prevalence rate of 0.1-2.3% (Pampaloni et al., 2022). However, differences among prevalence rates have been observed based on the demographic profile of the study populations.

1.2.2 Age Distribution

For adults with OCD, the reported average age of onset was 16.9 years according to a vast large transcontinental study. It also stated that the onset age for most cases was in between the range of 12-21 years (Brakoulias et al., 2017). Another recent study also reflects comparable findings concluding that about 60-70% of OCD population onset prior to the age of 25 with a mean age of onset of 19 years (Solmi et al., 2021). Provided the OCD patients get adequate treatment, youth have a more favorable prognosis than adults. A higher probability of recovery has been observed in adults with OCD when managed with proper health care, as 50% achieve remission within an average of 5 years follow-up time. Whereas, in youth the percentage is about 60% with adequate health care. On the other hand, without proper medical intervention there are higher chances of pediatric OCD being chronic (Cervin, 2023).

1.2.3 Gender distribution

The DSM-5 states that between the two genders, at the age of onset, males typically have a lower age than females. A pattern of variation of gender-related phenotypic expression of OCD has been observed with females found inclined towards cleaning, contamination dimension and greater comorbidity with impulse control disorders whereas symptoms such as forbidden thoughts, obsession with symmetry along with a greater comorbidity with Tourette syndrome are more evident in males (American Psychiatric Association, 2013; Pampaloni et al., 2022). Another study depicts that the incidence of OCD with varied gender distribution shows two peaks. The first peak of incidence is predominantly observed in males, within the range of 7-12 years along with a second peak near the age of 21, predominantly observed in females (Geller et al., 1998). Moreover, differences in the age of onset are also apparent with females demonstrating onset mostly after and males having onset before the age of 10. Again, amongst males the courses of onset are insidious and chronic. In case of females the courses of onset are more episodic with a possibility of worsening of OCD during pregnancy or postpartum increasing their susceptibility to developing OCD than other people (Pampaloni et al., 2022). Among females in the peripartum period, possible dysfunction of mother-infant relationship is evident due to OCD (American Psychiatric Association, 2013). A study reports that clinically significant symptoms of OCD are experienced by nearly 12% of perinatal women. While 2.3% and 1.7% of women are affected by perinatal OCD during pregnancy and postpartum respectively. It is assumed that the prevalence rates of perinatal OCD might be higher than reported because of the mental burden of possible negative consequences among women (Cervin, 2023).

1.2.4 Prevalence in Bangladesh

Prevalence of OCD differs based on the demographic of the study population (Algin et al., 2018). As reported by the National Mental Health Survey of Bangladesh, in 2018-2019, the percentage of Bangladeshi adults diagnosed with OCD was reported to be 0.7% (Chowdhury et al., 2020). A study concludes that the prevalence of OCD within the Bangladeshi population equals 1-3% of the population. The common age of onset is before 25 with presentations of extreme symptoms within more than 50% of the patients. Another study depicts that in this region the probability of OCD in adolescent boys is higher compared to girls, whereas the effect is somewhat equal for both genders among adults. Findings from another study in Bangladesh suggests that among OCD patients, females were more comorbid with higher lifetime diseases like hypothyroidism, skin disorder, diabetes and hypertension, while occurrence of co-morbid

anxiety disorders such as panic disorder, agoraphobia, social phobia etc. were more common among males. This study also indicated that the number of males were higher than females in case of onset before the age of 18. In contrast, a reverse phenomenon was observed after the age of 18, where females were more than males. Such patterns are consistent with the outcomes of global research regarding the higher prevalence of OCD in childhood for men with women exhibiting greater lifetime risks as adults (Algin et al., 2018).

Table 1. Summary of Global vs in Bangladesh Epidemiological Findings

Dimensions	Global Finding	Bangladesh Finding
Prevalence	2-3% of total population	1-3% of total population
	Adults with lifetime prevalence is 1.3%	Adults diagnosed 0.7%
	Here, the low percentage of diagnosis might indicate possible underdiagnosis or misdiagnosis.	
Age of onset	60-70% before 25 years	50% before 25 years
Relative age of onset by gender	Males onset earlier than females.	Males onset earlier than females. Consistent with global findings.
	Males have a higher probability of onset at adolescence.	Adolescent boys have a higher probability of onset at adolescence. Data consistent with global findings.

1.2.5 Suicide risk

OCD has historically been recognized to have a very low suicide risk which might have resulted in underestimation of its potential of causing suicide (Pampaloni et al., 2022). The DSM-5 denotes that about 50% of people suffering from OCD encounters suicidal thoughts throughout their lives with reports of suicide attempts evident in approximately one-quarter of the diseased population (American Psychiatric Association, 2013). Individuals with OCD have been evidenced to have a lifetime suicide attempt and lifetime suicidal ideation prevalence rate of 6%-51.7% and 26.3-73.5% respectively (Pampaloni et al., 2022). Notably, suicide risks are elevated in individuals with OCD when comorbidities i.e. major depressive disorders are present (American Psychiatric Association, 2013).

1.3 Comorbidity

It is worth mentioning that the presence of comorbid psychiatric disorders is widespread amongst OCD patients. According to the DSM-5th edition, about 76% of total OCD patients suffer from anxiety disorders, notably social anxiety disorder, panic disorder, generalized anxiety disorder and various phobias. People with OCD are more susceptible to developing other diseases listed within the OCRDs (American Psychiatric Association, 2013). A recent study states that comorbidity of minimum one mental disorder is observed among 60-90% of people with lifetime OCD. Depressive and anxiety disorders are the most common among the comorbid mental disorders with a prevalence of 50% in youth (Cervin, 2023). It has been evidenced that there is a significant association between OCD and some impulsive disorders like oppositional defiant disorder, stating that prevalence of such externalizing conditions among youth with OCD to be 15% (American Psychiatric Association, 2013; Cervin, 2023). Moreover, OCD has a higher rate in case of eating disorders and bipolar disorder (American Psychiatric Association, 2013). Another study summarizes the occurrence of the following comorbidities in OCD: social phobias 18%, simple phobia 22%, alcohol dependence being 14%, 12% panic disorder, eating disorders 17% and Tourette's syndrome 7% (Pampaloni et al., 2022). A summary of the possible comorbidities with their percentage is presented in figure 1 below.

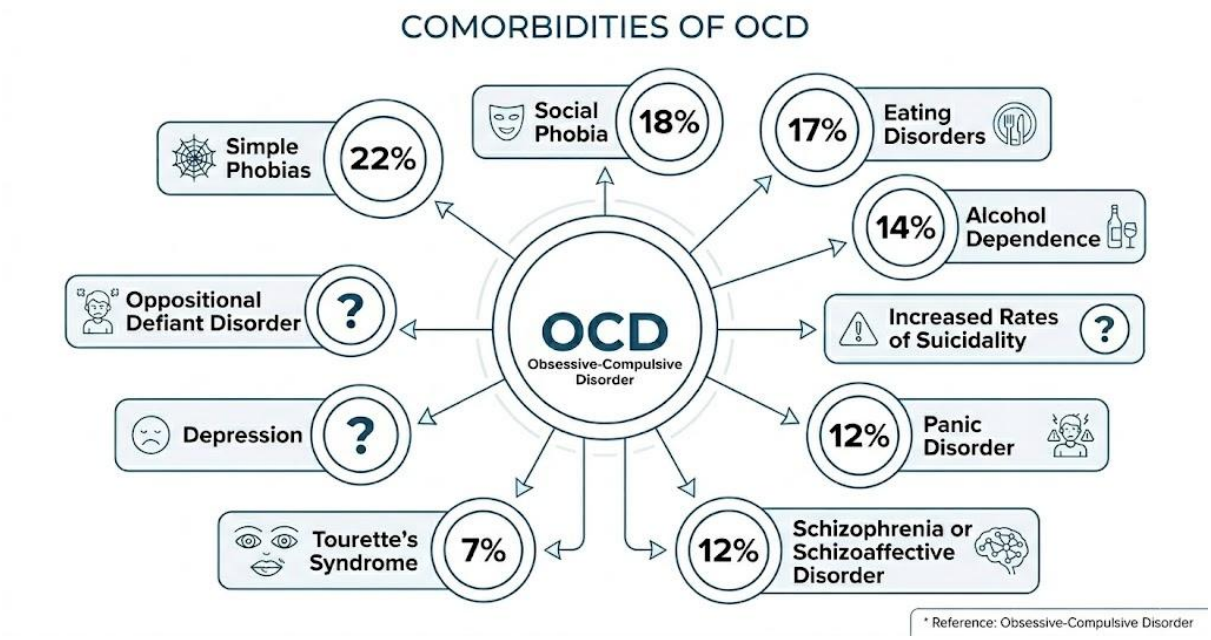


Figure 1: Comorbidities of Obsessive-Compulsive Disorder

This presence of comorbidities and similarity of symptoms of OCD has become one of the primary issues in diagnosis of OCD. As OCD might be underdiagnosed or misdiagnosed because of having similar symptoms. This risk of underdiagnosis or misdiagnosis highlights the importance of differential diagnosis in identifying OCD, which is also included in the DSM-5 diagnostic criteria for OCD.

1.4 Pathophysiology of Obsessive-Compulsive Disorder

The pathophysiology OCD is complex having multiple possible causes. Even though genetic and environmental factors were generally agreed as the main contributing factors behind the development and progress of OCD in individuals; Neurobiological dysfunction, neurochemical imbalances, autoimmune and inflammatory disorders have also been documented in the pathophysiology of OCD in individuals. All contributing factors to the pathophysiology of OCD as well as the hypothesis are represented in Figure 2 for better understanding.

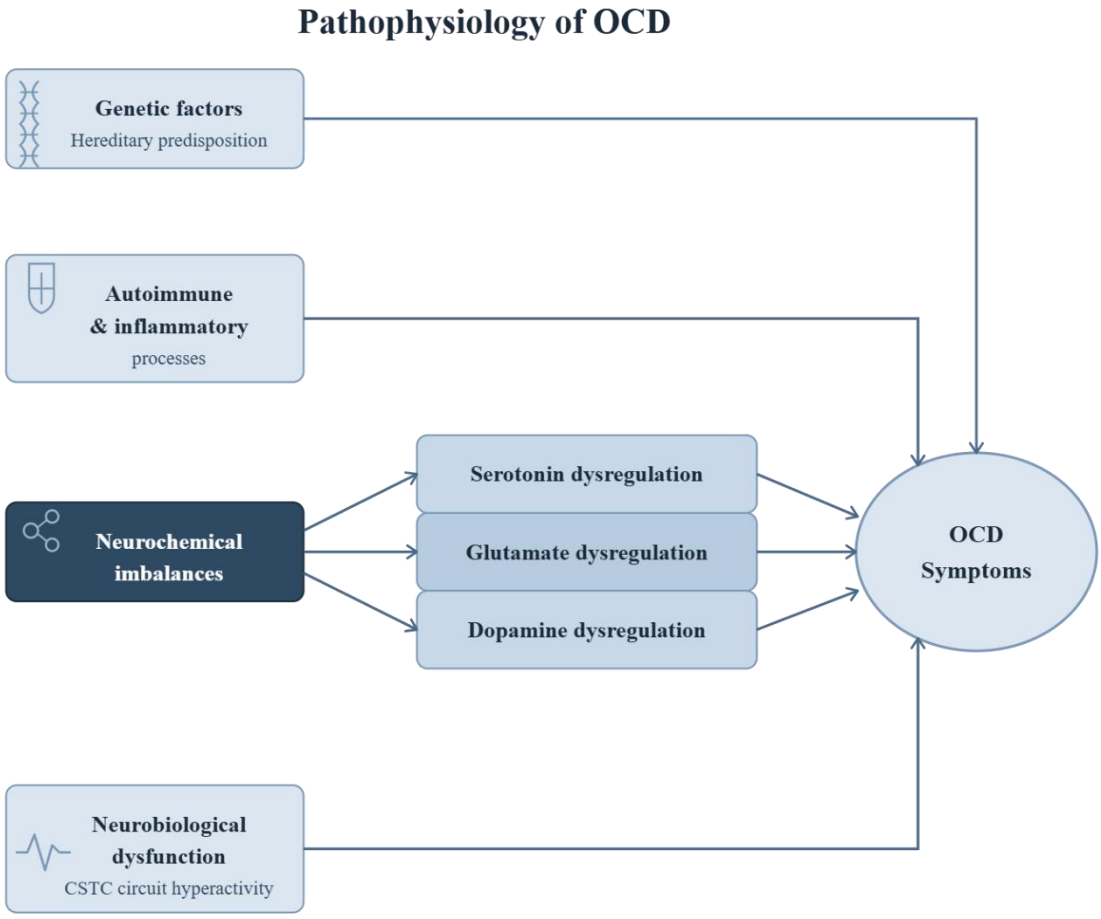


Figure 2: A Schematic Overview of the Possible Pathophysiology of Obsessive-Compulsive Disorder including the Hypotheses and Evidenced Mechanisms

1.4.1 Genetic Factors

OCD has been evidenced to be familial and heritable through different research studies concluding that genetic factors play a vital role. Most recently, a massive genome-wide association study discovered 249 effector genes for OCD along with 30 genome-wide significant loci, confirming the marked influence of genetics in OCD (Kurki et al., 2023). Previously, a study based in the Swedish National Patient Register indicated that approximately 35% of the risk of developing OCD was directly due to genetic factors. Whereas, the genetic and environmental maternal effect, that is, the mother's gene directly contributing to OCD risk by affecting the child's phenotype was found to be 7.6% of the total variance. Moreover, around

4-5% elevation in the effect of genetic factors in OCD risk was observed in cases of assortative mating i.e. mating between two individuals with OCD (Goodman et al., 2021).

1.4.2 Autoimmune and inflammatory processes

On another note, pathogenesis of OCD has also been associated with autoimmune and inflammatory processes dating back to 1998. A study concluded that Pediatric autoimmune neuropsychiatric disorder associated with streptococcus infection (PANDAS) is an autoimmune response that might lead to onset of OCD in childhood in many cases. This is an autoimmune reaction to infections caused by group A beta hemolytic Streptococcus (GAS). This study from 1998 later allowed for the expansion of research eventually identifying other pathogenic triggers for OCD besides GAS (Goodman et al., 2021).

1.4.3 Neurobiological dysfunction: Cortico-striato-thalamo-cortical circuit dysfunction

On the other hand, OCD caused by specific neurological etiologies have been observed in some cases. Pathophysiology of OCD is strongly linked to the cortico-striato-thalamo-cortical (CSTC) circuit dysfunction. The CSTC loop encompasses interconnected regions of the thalamus, cortex and striatum that are responsible for controlling behaviour. This includes the major cortical regions of prefrontal cortex (PFC) that are: anterior cingulate cortex (ACC), dorsolateral PFC (dlPFC), ventromedial PFC (vmPFC), and orbitofrontal cortex (OFC) linked to pathophysiology of OCD. The CSTC loop is featured by neural projections starting from parts of the cortex, then reaching the largest structure of the basal ganglia which is the striatum. The striatum consists of the nucleus accumbens (NAc) and serves as the main region for inputs. Following its exit from the striatum, the projection travels to the thalamus through an indirect referred to as inhibitory or direct referred to as excitatory pathway, passing through the basal ganglia. GPI/SNr are the main output regions of the basal ganglia that mostly conveys to the thalamus. The globus pallidus externa (GPe) and subthalamic nucleus (STN) are also some important structures within the basal ganglia. The flow of the direct pathway (excitatory pathway) from striatum is to the GPi/SNr. Whereas the indirect pathway (inhibitory pathway) reaches the GPi/SNr after passing through the GPe followed by the STN. Findings from several studies suggest the increased activity of the direct pathway with respect to the indirect pathway might be the reasons for some neuroimaging and clinical features of OCD. And this imbalance between the two pathways leads to recurrent obsessive and compulsive behaviour in an individual (Goodman et al., 2021). This CSTC circuitry can be simply understood from an

illustration visualizing the pathways and projections clearly included by Goodman et al. (2021) which has been attached in figure 3.

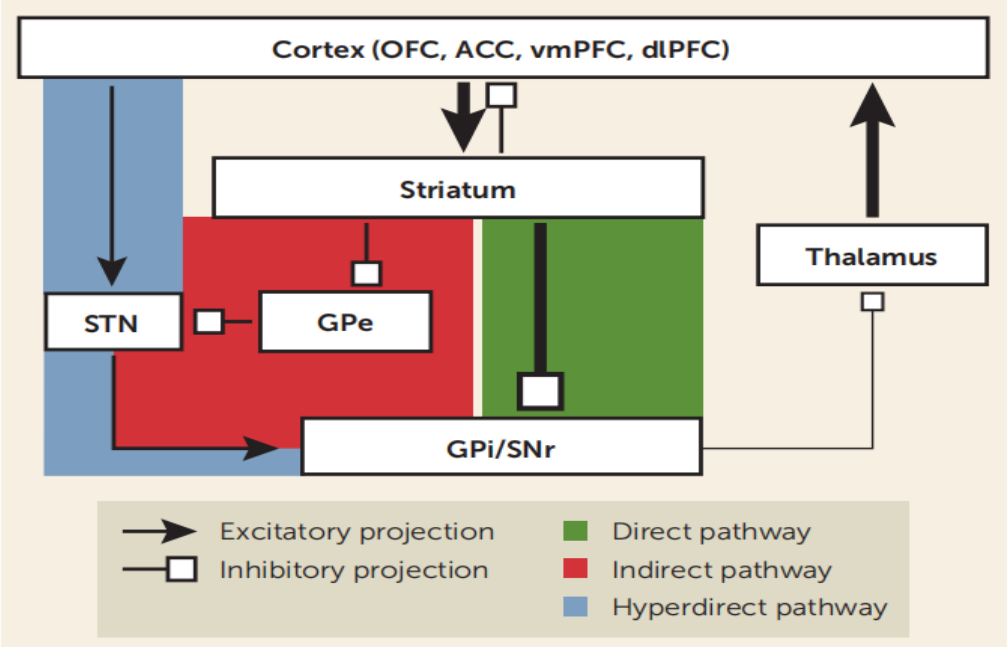


Figure 3: Schematic representation of the cortico-striato-thalamo-cortical circuit model for obsessive compulsive disorder (Goodman et al., 2021).

1.4.4 Neurochemical Imbalances

1.4.4.1 Serotonin dysregulation

The concept of serotonin dysregulation has been developed primarily based on clinical evidence of effectiveness. Following this multiple research and genetic findings have been conducted which suggest that OCD might be influenced by serotonergic neurotransmission associated gene alleles. There has not been any statistically confident specified allele or gene detected yet (Sinopoli et al., 2017). Another study reported that SLC6A4, the gene responsible for encoding 5-HTT or serotonin reuptake inhibitor; polymorphism influences the risk of OCD. A meta-analysis states a positive association of the risk allele with OCD affirming there is an increased risk of 25% for the carriers of the risk allele for manifestation of OCD (Pittenger, 2021).

As mostly risk associated polymorphisms in the promoter region are statistically similar for OCD and neuroticisms, considering their genetic similarities, the currently available pharmacotherapies and new targets that are effective in treating OCD might not be the specific cause responsible for causing OCD. Rather, they might be associated with broader and general psychiatric conditions, neuroticisms. The direct role of serotonin dysregulation therefore is still

uncertain, lacking enough statistically significant evidence. Some studies propose another debatable hypothesis relating OCD directly to the level of serotonin, stating serotonin imbalance is the root for OCD. Currently, there is no possible method for measuring serotonin levels in the synaptic cleft directly. As a result, no evidence has been found to support the hypothesis. Overall, even though serotonin reuptake inhibitors based pharmacotherapies can help half of patients, many still do not recover from this therapy. This signifies that serotonin dysregulation is not the only cause behind OCD. It is caused by a combination of different factors which require further research (Pittenger, 2021).

1.4.4.2 Glutamate theory

The recent advancements and latest findings from genomic studies, biochemical studies of cerebrospinal fluid, emerging imaging data and as well as implementation of animal models in establishing glutamatergic hypothesis of OCD. It suggests possible imbalance in the glutamatergic neurotransmitters in the CSTC loop that results in the development of OCD. Research has led to the optimization of various glutamate modulating agents to develop treatment for OCD. For instance- riluzole, troriluzole, ketamine, N-Acetylcysteine are some of the notable glutamate-modulating agents that have been studied in order to establish an effective pharmacotherapy. Studies encompassing these modulators are preliminary and sometimes inconsistent; no proven pharmacotherapy has been developed yet. Therefore, we can say that development of therapy targeted for the glutamate pathway serves as a promising direction for treatment of OCD (Goodman et al., 2021).

1.4.4.3 Dopamine dysregulation

Studies have not yet shown any significant consistency to indicate an association between dopamine and pathophysiology of OCD. So, the role of dopamine receptors is still unclear. However, dopamine dysregulation is evidenced in case of Tourette syndrome leading to a conclusion that OCD can be identified via dopamine dysregulation when it is comorbid with tic disorder. Otherwise, dopamine dysregulation's association with OCD remains vague (Pittenger, 2021).

1.5 Diagnosis

For diagnosis of OCD, psychiatrists mostly employ a semi-structured interview for evidence-based diagnosis. Semi-structured interviews mostly cover the DSM-5 criteria for diagnosis of the persistent repetitive behaviours (Lochner, 2024). The diagnosis test is followed by assessment of OCD with a severity assessment scale consisting of a set of questions that further

validates the diagnostic decisions. For severity assessment, the Yale-Brown Obsessive Compulsive Scale (Y-BOCS) is used in clinical settings.

1.5.1 DSM-5 Diagnostic criteria

The most common guidelines for the diagnosis of OCD are the DSM-5 criteria for diagnosis of OCD. This criterion addresses the obsessions and compulsions as the central defining features for identification of the disease. According to the DSM-5 criteria, obsession-compulsion must impair functionality, for example in social or occupational areas. These features must be time consuming and as well as cause clinically visible distress to be clinically labelled as OCD. It is necessary to assess that these clinical manifestations are not being induced due to any kind of illness, substance abuse or medication. Additionally, these clinical manifestations must not better align with the symptoms of any other mental disorder, i.e. differential diagnosis. Diagnostic specifiers include absent insight or delusional beliefs, fair, good or poor insight and whether the symptoms are associated with the history of any present or prior tic disorder (American Psychiatric Association, 2013).

1.5.2 Severity Assessment Tool: Yale-Brown Obsessive Compulsive Scale

The Y-BOCS was mainly developed for addressing and scoring the severity of OCD (Goodman et al., 1989). This scale is frequently employed by clinicians to assess the severity of OCD and to support diagnostic decisions. The Y-BOCS has been evidenced to have excellent internal consistency with a Cronbach's α coefficient of 0.89-0.96 (Storch, Rasmussen, et al., 2010). This signifies the high validity and reliability of the scale. It is a simple and cost-effective diagnostic tool that can be employed in a large variety of clinical settings.

The scale consists of 10 items. The total score is 0-40. Starting with no symptoms as 0, and extreme symptom as 4, each item can be scored from 0-4. Obsessions and compulsions can be scored separately from 0-4 for each item (Castro-Rodrigues et al., 2018; Goodman et al., 1989). It was revised to incorporate avoidance behaviour within its scoring. The Y-BOCS II was introduced. The Y-BOCS II also has high psychometric properties. To discriminate OCD with high sensitivity (85%-90%) and specificity (94-97%), the cutoff score was set at 13 in the Y-BOCS II. This scale can successfully discriminate controls from people with OCD and separate OCD from other anxiety disorders (Castro-Rodrigues et al., 2018). As a result, it is considered the golden standard for OCD assessment in clinical and research settings.

The Y-BOCS scale is also employed in the reassessment of OCD severity following treatments to track any possible progress. As stated by Hollander et al. (2010) a clinically significant

change is indicated if there is a Y-BOCS score difference of 5. Self-assessment through the Y-BOCS form is also widely used.

1.5.3 Diagnostic challenges

Even though OCD is the fourth most prevalent mental disorder globally, it is underdiagnosed and very frequently misdiagnosed due to its complex multifactorial nature (Pampaloni et al., 2022). The stigma regarding reporting of obsessive-compulsive behaviours, the diversity of its symptoms and as well as overlapping symptoms with other related mental disorders are some of the major causes behind such diagnostic challenges (Pampaloni et al., 2022; Singh et al., 2023). On the other hand, the presence of comorbid conditions in OCD further complicates the diagnosis process, often resulting in underdiagnosis or misdiagnosis. In case of pediatric individuals with OCD, diagnosis is frequently delayed due to the symptoms overlapping with other mental conditions. In many cases of OCD having sexual obsessions, the hesitation in acknowledging and reporting such behavior leads to the condition being underdiagnosed in the long term (Singh et al., 2023).

1.6 Treatment

Currently, in treatment of OCD, multiple approaches are used including various pharmacological interventions and behavioural therapies. Depending on the mechanisms and causative dysregulations of OCD, classes of various drugs have been studied. The current treatment approaches for OCD are briefly described.

1.6.1 Pharmacological Interventions

Due to the evidence of serotoninergic level changes being associated with the development of obsessive-compulsive symptoms, often resulting from a serotonin mediated increased postsynaptic signaling, selective serotonin reuptake inhibitors (SSRIs) were the first class of drugs that were implemented in the treatment of OCD (Del Casale et al., 2019). Drugs of this class including fluoxetine, sertraline, paroxetine and as well as voxamine have been found to be effective in treatment of OCD showing a positive dose response relationship (Reid et al., 2021). Typically, these drugs require prolonged use to show significant pharmacologic effect. It is recommended to continue use of SSRIs after symptoms of OCD have stopped to prevent possible chances of relapse. SSRIs are sometimes prescribed in combination with cognitive behavioural therapy to enhance the rate of success (Del Casale et al., 2019). A list of SSRIs and their evidenced success rates are as follows in a table below-

Table 2. SSRIs in Treatment of OCD

SSRI	Recommended dose	Evidenced pharmacological effect
Fluoxetine	40-60mg/day	Affects multiple OCD subtypes- reduces washing compulsion, improved quality of life, affects psychosocial functioning,
Citalopram	60 mg/day	Affective in refractory OCD. Improves quality of life, anxiety conditions, obsessive and compulsive behaviour and psychosocial functioning,
Paroxetine	20-60 mg/day	Its marked anxiolytic effect helps in treating OCD comorbid with anxiety and phobic disorders.
Fluvoxamine	100-300 mg/day	Prolonged use improved psychosocial skills and obsessive symptoms.
Sertraline	50-200 mg/day	Improves anxiety, phobia and avoidance behaviour associated with OCD.

Note. All the information contained within the table are from the article "Psychopharmacological Treatment of Obsessive-Compulsive Disorder (OCD)" by Del Casale, A., Sorice, S., Padovano, A., Simmaco, M., Ferracuti, S., Lamis, D. A., Rapinesi, C., Sani, G., Girardi, P., Kotzalidis, G. D., & Pompili, M. (2019). Psychopharmacological Treatment of Obsessive-Compulsive Disorder (OCD). *Current Neuropharmacology*, *17*(8), 710–736.

Aside from SSRIs, robust studies are ongoing regarding the effectiveness of drugs like clomipramine, Serotonin-Norepinephrine Reuptake Inhibitors (SNRIs) like venlafaxine milnacipran etc. in improving symptoms of OCD. These drugs show promising effectiveness in OCD treatment but are not clinically employed due to the lack of its consistency in clinical

safety and efficacy. Combination therapies of SSSRIs with clomipramine are considered effective second-line therapies (Del Casale et al., 2019).

1.6.2 Psychological Treatment

Effective psychological approaches to treat OCD include cognitive behavioral therapy (CBT) with exposure and response prevention (ERP). CBT addresses the major symptoms of OCD and evaluates the reasons for such behaviour which in turn assists the patients to control their activities. Even though the pharmacotherapies have proven efficacy, in children, CBT is much more recommended than the use of pharmacotherapies. This allows to prevent any adverse reactions or side effects caused by pharmacological interventions for that age group (Reid et al., 2021). ERP is a type of therapy where the patients are exposed to the factors that supposedly trigger their OCD symptoms and allows the patient to learn to suppress their response, that is, trying to control and resist their urge towards symptomatic behaviors. CBT using ERP has been evidenced to be more effective than any other psychotherapy such as psychoeducation and progressive relaxation therapy in management of OCD symptoms. As a first line pharmacotherapy, CBT with ERP is very effective (Reid et al., 2021).

1.6.3 Combination Therapy

In many cases, especially in cases of severe OCD, combination therapy of psychotherapy and psychopharmacological interventions are more promising than continuing with a single therapy (Skapinakis et ai., 2021). SSRIs are used in combination with CBTs as an effective treatment regime for adults with severe cases of OCD (Del Casale et al., 2019).

1.7 Chemokines and the CXCL Family

Chemokines, also known as chemotactic cytokines, are small proteins that are associated with immune and inflammatory responses. They initiate signaling via linking with G protein-coupled heptahelical chemokine receptors on cell surfaces. The role of chemokines in maintaining homeostasis of the immune system is pivotal. Chemokines can be classified into four subfamilies based on the precise configuration of the two cysteine closest to the N terminus in its structure (Hughes & Nibbs, 2018). The CXC family is one of them. CXC chemokines consist of a C-X-C motif, containing one uncertain amino acid between two cysteines. Further structural diversities can be denoted by the N-terminus ELR (Glu-Leu-Arg) motif. ELR+CXC are angiogenesis activators: CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8, CXCL17. And CXCL4, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14 and

CXCL16 that belong to ELR-CXC chemokines which are angiogenesis inhibitors (Zhou et al., 2023).

The components of the CXC family have been associated with the pathophysiology of multiple diseases including different types of cancer and inflammatory diseases. For instance, breast cancer, ovarian cancer, hepatocellular carcinoma etc. are influenced by the effect of CXCL chemokines in the growth of neoplasm. This makes CXCL chemokines a potential candidate for the development of targeted pharmacotherapies (Zhou et al., 2023).

1.8 Chemokine (C-X-C Motif) Ligand 4

Chemokine (C-X-C motif) ligand 4, CXCL4 also known as platelet factor 4 (PF4) is a member of the C-X-C chemokine family (Zhou et al., 2023). Anti-heparin and anti-angiogenic properties and as well as maintaining hemostasis and immune cell stimulation at tumor sites are some of its established key functions (Ji et al., 2025; Zhou et al., 2023). In mature platelets of healthy individuals, 10% of the total volume is attributed to alpha (α) granules. These appear to be round or oval granules with a diameter of 200-500nm, with an approximate count of 50-80 per mature platelet (Yadav & Storrie, 2017). Numerous proteins, antimicrobial peptides, RNA and growth factors, are stored within the α granules (Manne et al., 2017). These α granules also contain CXCL4 or PF4 (Wang et al., 2019). Additionally, the presence of specialized α granules termed as the storage granules are responsible for the synthesis of CXCL4. CXCL4 is present at a very high concentration within platelets with an abundance of 20μg per $1\times10_{9}$ platelet cells and 2-10 ng/mL in plasma (Yadav & Storrie, 2017). Depending on P-selectin, these chemokines are released from activated platelets after activation and impact a wide range of cells. Some recent studies concluded that macrophages along with activated T-cells are responsible for synthesis of CXCL4 even though previously, activated platelets were considered as the only synthesis site. The specific function of the chemokines in macrophages and T-cells is still undiscovered (Shi et al., 2014; Wang et al., 2019).

1.8.1 Structure of Protein

CXCL4 has a 101 amino acid structure containing two histidine and a single tyrosine along with four cysteine residues. This structure forms two disulfide bonds without any tryptophan, methionine residue, the monomer contains three anti-parallel β-strands: Pro37-Lys46, Gly48-Leu53 and Thr25-Gly33 that are hydrophobic in nature, a single glutamate rich amino terminal aperiodic sequence: Asp7-Glu18 and carboxy-terminal α-helical amphipathic structure: Ala57-Ser70. Two asymmetric CXCL4 dimer forms a stable cylindrical tetramer (Wang & Huang,

2013). A tetrachromatic crystal molecular structure of CXCL4 tetramer by Zhang et al. (1994) has been included in figure 4 for easier comprehension. Each colour in figure 4 represents the structure of a single monomer of CXCL4 (Zhang et al., 1994).

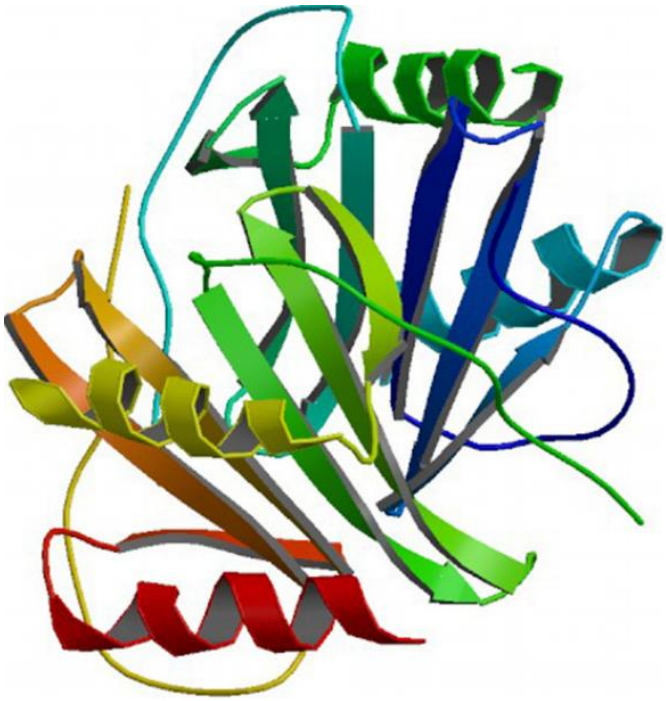


Figure 4: 3D crystal molecular structure of CXCL4 tetramer (Zhang et al., 1994).

1.8.2 Biological Role in human body

CXCL4 is a multifunctional protein which plays a diverse role in the biological functioning of the human body. Its major functions encompass interference of platelet coagulation, promoting inflammation, hematopoiesis inhibition and as well as angiogenesis inhibition. CXCL4 has been found to be antiangiogenic ELR-negative chemokine, showing inhibitory effects on key angiogenic growth factors. CXCL4 acts as a negative modulator of megakaryopoiesis (Wang & Huang, 2013). On the other hand, it plays an important role in receptor independent immune cell recruitment by binding to proteoglycan's sugar glycosaminoglycan (GAG), which also contributes to increasing vascular permeability. It exerts its effects by binding to GAG within the extracellular matrix, without binding to any specific chemokine receptor (Gray et al., 2023). Moreover, it influences vascular remodeling during atherosclerosis or injuries, via regulating gene expression, proliferation, cytokine release and migration (Kaczor et al., 2022). Other notable functions include mediating megakaryocyte maturation, neutrophil adhesion, promoting B cell differentiation, neutrophil adhesion and as well as T cell differentiation (Ji et al., 2025). CXCL4 contributes to its diverse range of activities and is also an important factor

in pathogenesis of multiple diseases, including fibrotic and inflammatory disorders by altering toll-like receptor (TLR) responses profiles (Yang et al., 2022).

1.8.3 Mechanism of action

1.8.3.1 General Release Mechanism

CXCL4 is released by activated platelets via platelet aggregating agents. Agents like arachidonic acid, thrombin or adenosine 5 diphosphate stimulate the release of CXCL4 from α granules of platelets (Wang & Huang, 2013).

1.8.3.2 Mechanism of Actions in Platelet Coagulation

CXCL4 has been evidenced to have both pro and anti-coagulant functions. A cascade of interrelated proteolytic reactions is instigated by CXCL4. During coagulation, thrombin is responsible for the conversion of fibrinogen to fibrin that triggers platelet activation leading to coagulation. CXCL4 exerts its procoagulant effect as it blocks acceleration of thrombin inactivation by preventing the complex formation of heparin with anti-thrombin III. Thus, the heparin facilitated thrombin activation is inhibited. Aside from heparin, it also affects other coagulation factors. For instance, it blocks coagulation factor XII (Hageman factor) activation and as well as activated protein C generation. As activated protein C has an inhibitory effect on clotting factors Va and VIIIa, it can be concluded that CXCL4 provides an anti-coagulant effect by stimulating protein C generation (Wang & Huang, 2013).

1.8.3.3 Mechanism of Immune Cell Recruitment

Chemokines usually act through binding with classical chemokine receptors. CXCL4 has been evidenced to mediate immune cell recruitment without binding to a chemokine receptor. Rather it depends on extracellular matrix interactions. Gray et al. (2023) has described this unique mechanism of action of CXCL4 compared to typical chemokine interaction along with a detailed illustration, which has been included in figure 5 for easier understanding and visualization.

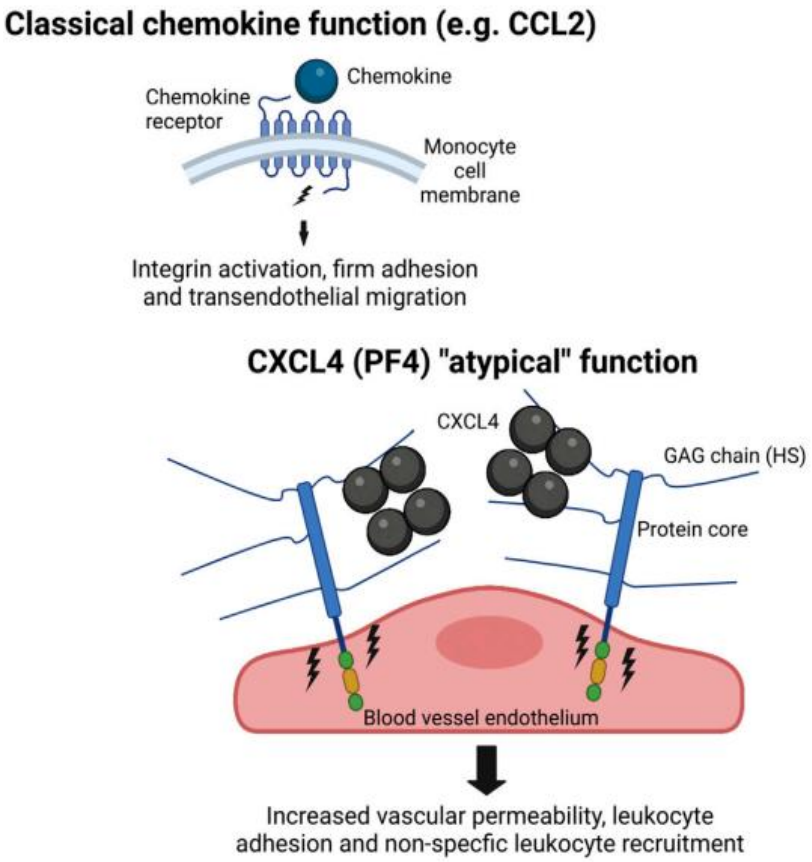


Figure 5: Mechanism of action of CXCL4 in immune cell recruitment in comparison with typical chemokine interaction with specific chemokine receptors (Gray et al., 2023).

It binds to GAG sugars present on the surface of proteoglycans. This binding within the endothelial matrix on proteoglycans results in the localization of CXCL4 on the endothelial surface, enhancing leukocyte adhesion and transmigration. On the other hand, the selectivity and localization of CXCL4 is influenced by the sulfonation patterns of GAG, hence affecting its immune cell recruitment. Non-receptor dependent immune cell recruitment and a marked increase in vascular permeability is triggered (Gray et al., 2023).

1.8.3.4 Mechanism of Hematopoiesis Inhibition

CXCL4 inhibits megakaryocyte progenitor cell maturation and proliferation, eventually acting as a negative modulator of megakaryopoiesis. Hematopoietic progenitor cells (HPCs) contain a chondroitin-sulfate moiety. CXCL4 interacts with this specific moiety on the HPCs causing an increase in adhesion and quiescence of progenitors. Thus, it influences hematopoiesis directly (Wang & Huang, 2013).

1.8.3.5 Mechanism of Angiogenesis Inhibition

Angiogenesis is inhibited by CXCL4 by suppressing endothelial cell proliferation and migration. These are mediated by numerous growth factors. Vascular endothelial growth factor (VEGF165) and basic fibroblast growth factor (bFGF-2) are two angiogenic growth factors which are responsible for inducing endothelial growth, cell differentiation and migration. CXCL4 interferes with the mitogenic pathway of bFGF-2 and VEGF165 via two possible mechanisms. CXCL4 either directly binds with bFGF-2 interfering with high affinity receptor binding by preventing internalization and demerization. It can also bind to VEGF165 directly on the cell surface and interferes with angiogenic signaling. On the other hand, it can indirectly inhibit receptor binding of VEGF165 and bFGF-2 by disrupting the effect of Heparan sulfate proteoglycans (HSPGs). As HSPGs are responsible for binding and stabilizing the formed receptor complexes, disrupting it will also block receptor binding exhibiting antiangiogenic properties. Additionally, CXCL4 can inhibit cell cycle progression in the S phase disrupting DNA synthesis and initiate apoptosis via activating CXCR3B receptor isoform on endothelial cells. Thus, it exerts its angiostatic effects. Moreover, CXCL4 can act as an antagonist for integrins to their ligands, interrupting adherence to endothelial cells during angiogenesis. CXCL4 can further impact the signaling pathway of angiogenesis by interacting with certain factors- interleukin (IL-8) and VEGF121 (Wang & Huang, 2013). A summary of the major pathways involving CXCL4 contributing to angiogenesis inhibition are summarized schematically in figure 6.

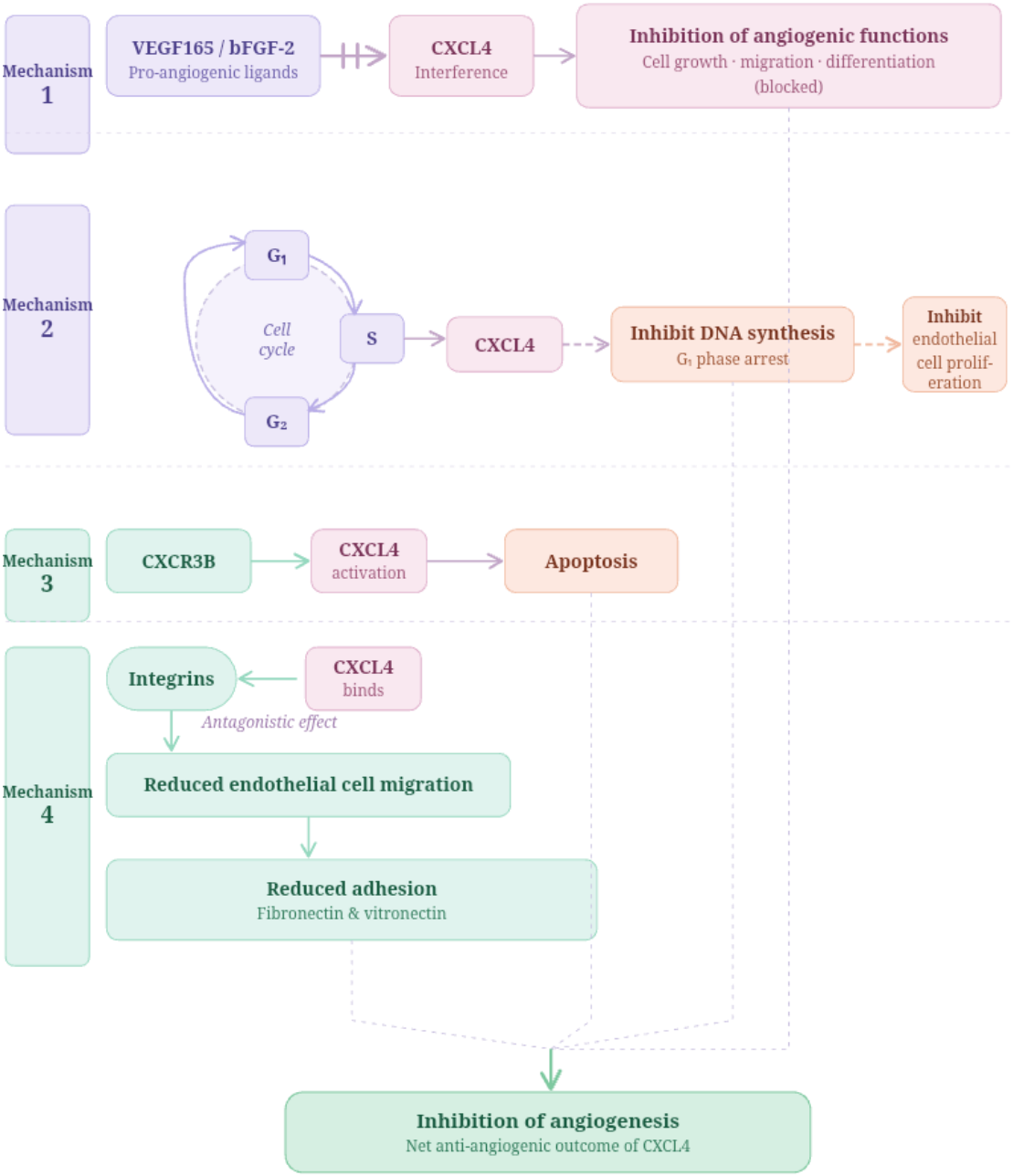


Figure 6: Mechanisms of action of CXCL4 contributing to angiogenesis inhibition

Thus, in the mechanisms, CXCL4 can interact with more than one target exerting its antiangiogenic effect.

1.8.4 CXCL4 as a Potential Biomarker

Till date, CXCL4 has been associated with the pathophysiology of multiple diseases. It has been linked with the development of different types of cancer. Along with CXCL14, CXCL9

and CXCL12, CXCL4 has been reported to be correlated with breast cancer (Hozhabri et al., 2022). Moreover, it has also been reported that levels of CXCL4 changes have been observed in case of lung fibrosis. Significantly high levels of CXCL4 in systemic sclerosis concludes it as a potential biomarker for systemic sclerosis (van Bon et al., 2014).

So far, the relevance of CXCL4 as a biomarker in the pathophysiology of OCD still remains unclear as no research studies have been conducted exploring the levels of CXCL4 in OCD patients. Owing to the fact that immune and inflammatory dysregulation are some of the factors that are linked to OCD, no specific single biomarker behind this pathophysiology has been discovered yet (Bhatt et al., 2024). In such circumstances, by considering the potential effect of CXCL4 in the pathophysiology of OCD might help to distinguish it as a potential biomarker. CXCL4 is a strong inflammatory mediator that works in a multifaceted manner. One of its most notable mechanisms include receptor independent immune cell recruitment (Gray et al., 2023). A study has concluded elevated levels of various proinflammatory biomarkers including IL-1β, TNF-α, IL-6 and IL-8 in OCD patients with respect to that of healthy individuals (Rodríguez et al., 2017). Several other studies have also explored these inflammatory biomarkers but due to the inconsistency between their levels, no biomarkers have been established as a clinically relevant tool for OCD diagnosis. Considering the proinflammatory effect exerted by CXCL4, and by studying the levels of CXCL4 in OCD compared to controls, it is possible to propose CXCL4 as a clinically relevant biomarker.

1.9 Rationale of The Study

Currently, there are no specific biomarkers that can be quantified specifically for identification of OCD. As OCD is a polygenic disorder, it cannot be attributed to one single biomarker or a single causative factor. The possible scope of diagnosis gets complicated by the presence of overlapping symptoms with other similar mental disorders, eventually limiting development of targeted therapies. At present, therapeutic intervention through SSRIs is the only available medication for this condition. SSRIs do not provide any definitive cure.

In such circumstances, by exploring the levels of CXCL4 among OCD patients, it might be possible to identify an association. This association can be beneficial as a supporting tool for OCD diagnosis, as CXCL4 levels can be easily quantified with the help of enzyme linked immunosorbent assay (ELISA). On the other hand, following a possibly established association between CXCL4 levels and OCD, further research might be able to associate CXCL4 levels with OCD severity, enhancing clinical utility.

Moreover, studies relating to the establishment of association of CXCL4 and OCD might provide new pharmacotherapeutic approaches for development of targeted therapy surrounding CXCL4 mediated pathways. Thus, investigating CXCL4 as a potential marker provides scopes for improving diagnostic precision, impact future pharmacotherapy development and as well as provide insights into the underlying pathophysiology of OCD.

1.10 Aim and Objective of The Study

The study aimed to determine the serum CXCL4 levels in OCD patients and healthy controls (HCs) among the Bangladeshi population. Assessment and establishment of any possible association of the biomarker with OCD might help in the diagnosis of OCD and as well as introduce methods of developing new pharmacotherapeutic approaches.

Chapter 2

Materials and Methods

2.1 Study Populations

This study is an observational case-control study that was conducted with 376 participants. 200 OCD patients were enrolled in this study from the National Institute of Mental Health (NIMH) and Hospital, Dhaka Bangladesh. The mean age of OCD patients were 28.66 years (SD =9.14). Additionally, 176 HCs with mean age 29.97 years (SD= 8.925) were enrolled from different areas in Bangladesh. The HCs were enrolled carefully considering the gender, age and demographic similarity with the OCD patients. Sample collections for HCs were done across the country.

2.2 Sample Collection

2.2.1 Ethical Consideration

The ethical approval for this project (SEU/Pharm/CECR/024/2024) was obtained from the committee for Ethical Compliance in Research, Southeast University Bangladesh. The entire process was carried out aligning with the aspects of the Helsinki Declaration. The whole investigation was conducted considering the ethical issues carefully and a criterion was set to uphold the ethical values throughout the process of the study. The criteria considered are as follows-

i. Written consent and all the information collected were solely obtained by the voluntary participation of the individual without any coercion.
ii. The confidentiality of all the participants was maintained carefully.
iii. Appropriate permission was obtained from an established Research Ethics Committee to conduct this study.
iv. No intervention was done to get any desired outcome,
v. It was made sure the research did not affect the treatment progress for any of the patients.
vi. The participants were instructed to contact the investigators in case of any confusion.
vii. The details presented to the individuals prior to getting their written consent were-
    a) A brief overview of the purpose and nature of the study.
    b) Clarification that participation is solely voluntary. The participants have the right to refuse.

c) No financial benefits were offered to the individuals for their participation.
d) Details on the procedure and duration of study.
e) All information about the possible benefits and hazards were provided.
f) No body organ or tissue were involved in this research.

All data obtained were only utilized for research purposes.

2.2.2 Consent Form

Before collecting blood samples, information and enrolling study participants, written consent was essential. Information and sample collection started only after the written consent form was obtained. The consent form is attached to appendix A.

2.2.3 Selection Criteria

This case-control study only contained Bengali speaking adults. All the 200 patients enrolled were clinically diagnosed with OCD by a certified psychiatrist. All the information was collected, and the participants were enrolled based on certain inclusion and exclusion criteria. A structured interview was conducted with the help of a questionnaire in accordance with the DSM-5 to recruit the patients. And the severity assessment for all patients was done by using the Y-BOCS scale. In the case of enrolling 176 HCs, a similar method was used employing a questionnaire accompanied by the Y-BOCS scoring scale.

2.2.3.1 Inclusion Criteria

i. Age range acceptability: 18-60 years.
ii. Both male and female
iii. Applicable marital status: both married and unmarried
iv. Eligibility of OCD patients required evaluation by certified psychiatrist
v. Individuals who volunteered to provide their information and blood sample

2.2.3.2 Exclusion Criteria

i. Patients with diagnosed acute or severe medical conditions
ii. People unwilling to provide blood samples
iii. People with cognitive impairment
iv. People with preexisting medical conditions require medications that can impact the levels of CXCL4.
v. People with comorbid psychiatric illness or mental retardation.

vi. People with past history of alcohol consumption, drug dependence or tardive dyskinesia caused by neuroleptics, excessive weight, severe physical ailments, and infections.

2.2.4 Procedure of Data Collection

Blood samples and data were collected from the OCD patients after thorough assessment of OCD by a certified psychiatrist. Each patient and HC were enrolled by following the inclusion and exclusion criteria. Followed by this, a detailed questionnaire form was used to collect socio-demographic information from the ideal candidates. 5mL of blood was withdrawn from the cephalic vein of the enrolled candidates. A detailed overview of the collection process is as follows:

i. Greetings
ii. Following verification of diagnosis, a brief explanation of the study and ethical issues were given to the patients.
iii. A mandatory consent form was provided to the patients.
iv. Data was obtained by direct interviewing of the patients at the department of psychiatry.
v. All inclusion and exclusion criteria were verified.
vi. Socio-demographic data were collected using a structured questionnaire.
vii. With the help of an expert, a 5 mL blood specimen was drawn from the cephalic vein of each participant.

For each participant, about 30 minutes were required for completing sample and data collection.

2.2.5 Questionnaire Form for Data Collection

Comprehensive data including sociodemographic, biological and clinical findings from all the participants were collected with the help of a questionnaire form. The questionnaire form is attached in appendix B. For OCD patients, the Y-BOCS scale was used to determine severity after interviewing by the questionnaire. The Y-BOCS scale is attached in appendix C.

2.2.6 Accessories Required for Sample Collection

Materials used in blood sample collection from the participants are listed in the following table 3

Table 3: Materials used in Blood Sample Collection

Materials	Specifications
Disposable syringe	5ml
Band-Aid	
Tourniquet	
Butterfly needles	
Hexisol	50ml
Sterile cotton	
Falcon Tubes	15ml
Eppendorf Tube	1.5 mL
Ice-Box	

Figure 7: Hexisol, alcohol pads and band aid.

Figure 8: Ice Box and Falcon tubes

2.2.7 Blood Sample Collection from Participants

A 5 mL disposable syringe was used to collect 5 mL blood from 176HCs and 200 OCD patients. Blood was withdrawn from the cephalic vein of the participants and collected in a falcon tube. The collected samples were preserved with the help of an ice box.

2.2.8 Preparation of Serum and Storage

Followed by the collection, blood samples were allowed to clot in the falcon tubes. The tubes containing the clotted blood were centrifuged at 3000 rpm for 15 minutes approximately at room temperature in order to separate the blood cells and the serum. A clear supernatant was obtained after centrifugation. The serum was then separated carefully and was transferred to Eppendorf tubes. The Eppendorf tubes containing the serums were refrigerated at -80℃ for optimal preservation and further analysis.

Figure 9: Refrigerator for sample storage

Figure 10: Centrifuge machine for serum separation

2.3 Materials Required for the Analysis of Serum CXCL4

For determination of serum concentration of CXCL4, Human CXCL4/PF4 ELISA Kit PicoKine by Booster Bio, USA was used.

Table 4: Materials used in analysis of serum CXCL4

Apparatus/ Materials	Specifications
Micropipette tips	10 µL -100 µL
Micropipette tips	100 µL -1000 µL
Micropipette	10 µL -100 µL
Micropipette	100 µL -1000 µL
Volumetric flask	500 mL
Eppendorf tube	1500 µL
Multichannel micropipette	50 µL -300 µL
Microplate Reader	
Incubator	

Figure 11: Incubator

Figure 12: Microplate reader

Figure 13: Eppendorf tube

Figure 14: Multichannel micropipette

(50 µL -300 µL)

2.4 Sample Analysis

An enzyme-linked immunosorbent assay (ELISA) kit was used to measure the serum concentration of CXCL4 for analysis of the sample.

2.4.1 Required Reagents for Serum CXCL4 Analysis

Table 5: Required Reagents for Serum CXCL4 Analysis

Description	Quantity/ Volume
Microplates	5
Plate Sealers	20
Human PF4 Standard	10 ng/vial
Rabbit anti-human CXCL4 polyclonal antibody	500 µL /vial
Goat anti-human CXCL4 polyclonal antibody (capture antibody)	500 µL /vial
Avidin-Biotin-Peroxidase Complex (ABC)	500 µL /vial
Reagent Diluent (10)	20 ml/vial 2
Stop Solution	10 ml/vial 5
Colour Developing Reagent	10 ml/vial 5
PBS (20)	25 ml/vial 2
PBS-T (20)	25 ml/vial 2

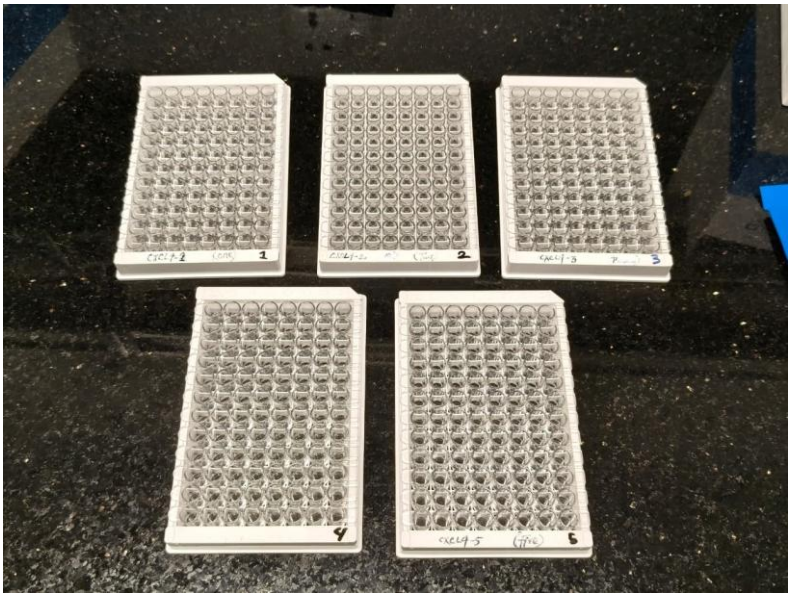


Figure 15: 96 Well Microplates

Figure 16: Reagent Diluent (10x)

Figure 17: Stop Solution

Figure 18: Colour Developing Reagent (TMB)

Figure 19: PBS (20x)

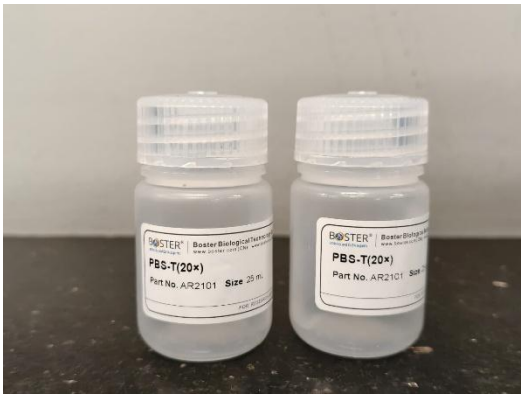


Figure 20: PBS-T (20x)

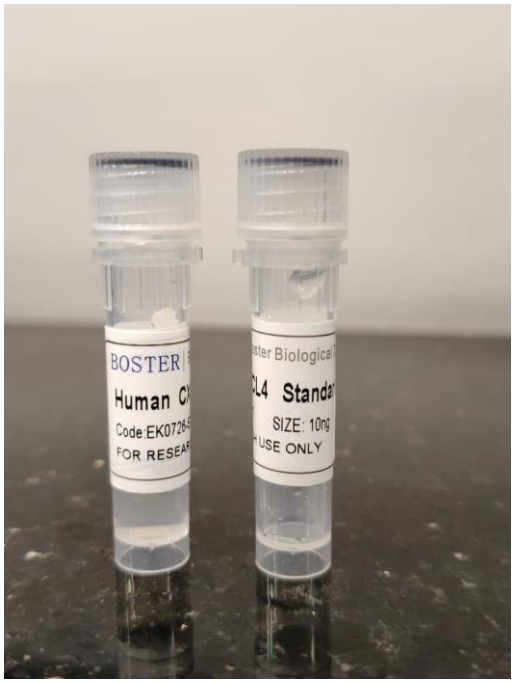


Figure 21: Human PF4 Standard

Figure 22: Goat anti-human CXCL4 polyclonal antibody (Capture antibody)

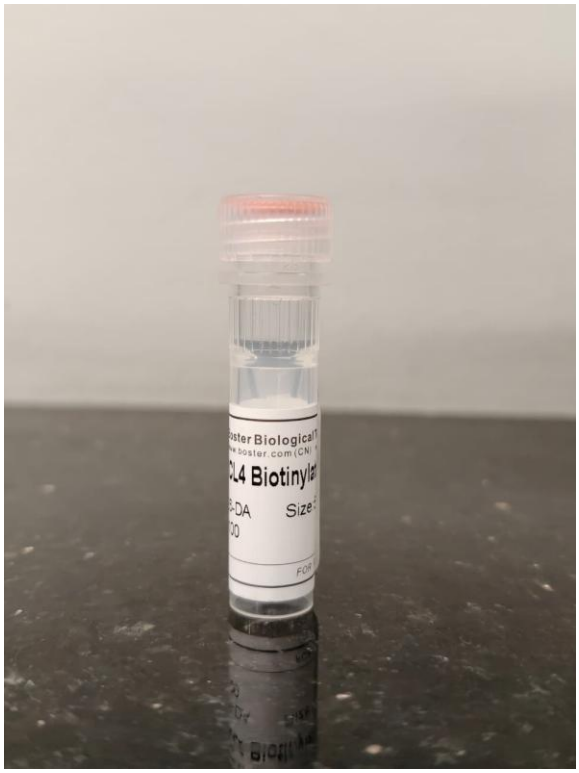


Figure 23: Detection antibody

Biotinylated Goat anti-human CXCL4 polyclonal antibody

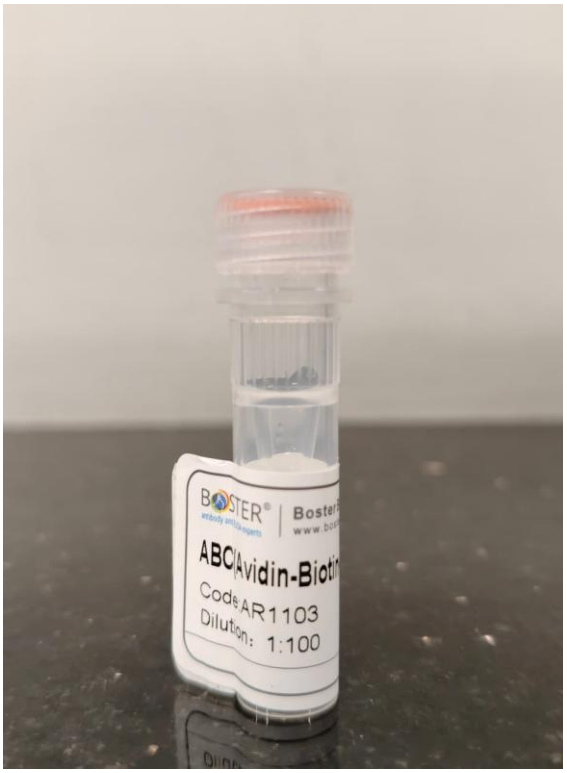


Figure 24: Avidin-Biotin-Peroxidase Complex

2.4.2 Preparations Required for CXCL4 Assay

The reagents provided were brought to room temperature and were used immediately to prepare the required working solutions.

2.4.2.1 Wash Buffer

PBS wash buffer: The provided PBS solution was used to prepare the wash buffer by diluting the provided amount. 25mL of PBS (20x) was transferred to a volumetric flask, volume adjustment was carried out by adding distilled water up to the 1L mark.

PBS-T wash buffer: The PBS-T (20x) wash buffer was prepared in the same way as PBS wash buffer. 25 mL of the supplied PBS-T (20x) was transferred to a volumetric flask; volume adjustment was carried out by adding distilled water up to the 1L mark.

2.4.2.2 Plate Preparation

i. Capture antibody diluent was used to dilute capture antibody in 1:100 ratio. That is, 500 µL provided capture antibody was diluted with 49.5mL diluent. A 96-well microplate was immediately coated with the diluted capture antibody. 100 µL of capture antibody was used per well. The plate was sealed and stored. It was stored at 4°C.

ii. Then after removing the cover the liquid was discarded. The plate was gently blotted on a paper towel on the table to get rid of any liquid remaining. It was not allowed to completely dry.

iii. Incubation for 2 hours at room temperature after reagent diluent 200 µL addition to the microplates.

iv. Each well of the plate was drained of liquid and washed with PBS three times. The process was carried out by filling each well with 300 µL PBS and then inverting and gently blotting the plate on a paper towel to get rid of excess liquid.

v. The procedure I to IV was repeated 4 more times to coat the remaining 4 microplates.

2.4.2.3 Reconstitution of Human PF4 Standard

For each experiment, standard preparation required 10 ng of lyophilized human CXCL4 standard. At first, the standard was gently spun. 1 mL of reagent diluent was added to make a 10 ng/mL concentration. The standard stock was allowed to set for about 10 minutes with continuous agitating gently.

Serial dilutions were carried out with the standard stock to prepare dilutions in tube 1 , tube 2, tube 3, tube 4, tube 5, tube 6, tube 7 and tube 8 with concentrations 10,000 pg/mL (Std-1), 5000 pg/mL(Std-2), 2500 pg/mL (Std-3), 1250 pg/mL (Std-4), 625 pg/mL (Std-5), 312 pg/mL (Std-6), 156 pg/mL (Std-7) and 0.0 (Std-8) respectively. The tube 8 was used as blank for all the experiments.

2.4.2.4 Rabbit Anti-human CXCL4 Polyclonal Antibody Solution Preparation

500 µL Rabbit Anti-human CXCL4 Polyclonal Antibody was provided in each vial. This solution required immediate preparation prior to use. To prepare the working solution, a 1:100 dilution was carried out. 49.5mL capture antibody diluent was added to the provided volume of CXCL4 Polyclonal antibody and was thoroughly mixed to prepare the required Antibody solution.

2.4.2.5 Working solution Preparation: Biotinylated goat anti-human CXCL4 polyclonal antibody

The biotinylated goat anti-human CXCL4 polyclonal antibody (detection antibody) was 500 µL. Immediate preparation was required prior to use. 49.5mL reagent diluent was added to the provided volume of detection antibody and was thoroughly mixed to prepare the detection antibody working solution. A 1:100 dilution was carried out to get the required solution. It was prepared immediately before use.

2.4.2.6 Working solution Preparation: Avidin-Biotin-Peroxidase Complex (ABC)

The provided Avidin-Biotin-Peroxidase Complex (ABC) was 500 µL. A 1:100 dilution was carried out to get the required solution. 49.5mL reagent diluent was added to the provided volume of ABC and was thoroughly mixed to prepare the ABC working solution. It was prepared immediately before use.

2.5 Microplate Designs for Assay Procedures CXCL4

Five 96 well microplates were used for analysis of serum CXCL4. All the microplates had the same distribution of standard, patient and HC samples. The plates were prepared following the design 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	Blank	P	P	P	P
B	P	P	P	P	P	P	P	P	P	P	P	P
C	P	P	P	P	P	P	P	P	P	P	P	P
D	P	P	P	P	P	P	P	P	P	P	P	P
E	P	P	P	P	C	C	C	C	C	C	C	C
F	C	C	C	C	C	C	C	C	C	C	C	C
G	C	C	C	C	C	C	C	C	C	C	C	C
H	C	C	C	C	C	C	C	C	C	C	C	C

Figure 27: Microplate Design for CXCL4 Assay

Here, P stands for patients, and C stands for healthy control of the study population.

2.6 Assay Procedure of CXCL4

i. All working standards were prepared.

ii. The microplate strips were removed and 100 µL of standard, sample and blank were loaded into the well according to the plate designs.

iii. The wells were covered with a sealing strip and the plate was incubated at room temperature for 120 minutes.

iv. After removing the cover, the liquid was discarded. The plate was gently blotted on a paper towel on the table to get rid of any liquid remaining. It was not allowed to completely dry.

v. Each well was filled with 100 µL of biotinylated goat anti-human CXCL4 polyclonal antibody followed by incubation at room temperature for 90 minutes after it was sealed with a sealer.

vi. The plate was washed 3 times with PBS. 300 µL microliter was used per well each time followed by discarding, blotting and then again washing.

vii. The plate was again incubated for 40 minutes at room temperature after filling each well with 100 µL Avidin-Biotin-Peroxidase Complex (ABC).

viii. The plate was washed 5 times with PBS-T in similar procedure as the previous washes.

ix. 90 µL of colour developing reagent was added to each well and the plates were kept in dark for 30 minutes.

x. After 30 mins all the wells had formed a blue colour, especially darker in the top 4 standards. 100 µL stop solution was added to each well which resulted in an immediate colour change to yellow.

xi. The absorbance was recorded with a microplate reader at 450nm. Absorbance was recorded within 30 minutes of adding stop solution.

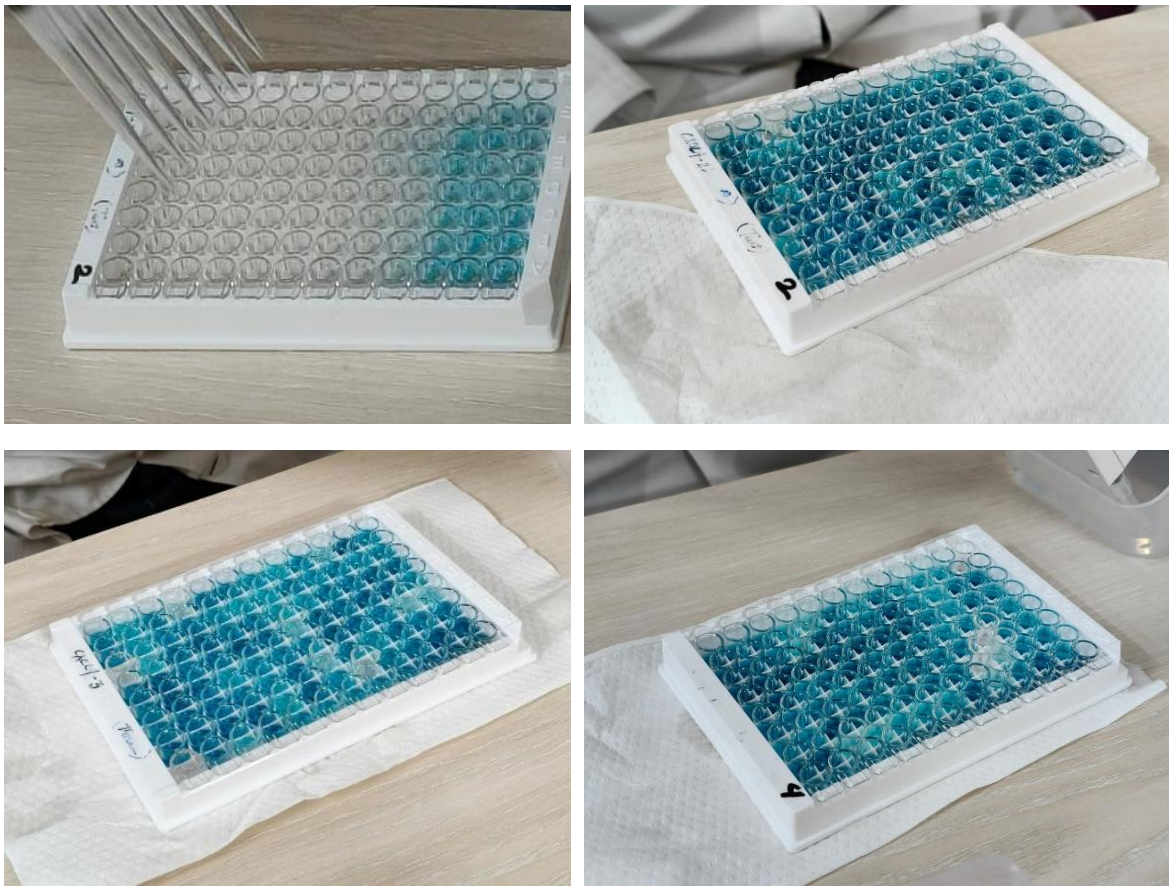


Figure 26: Microplates During and After Addition of Colour Developing Reagent

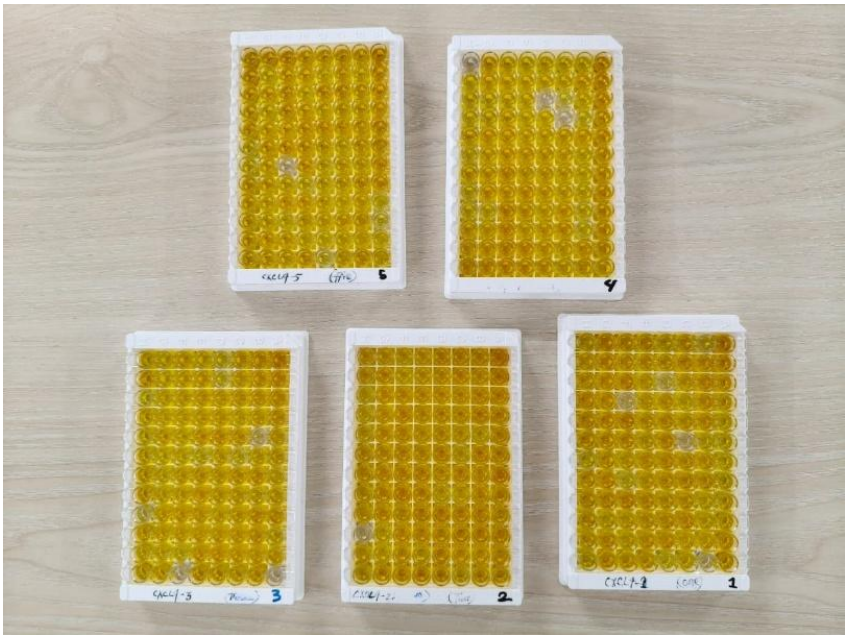


Figure 27: Microplates After Addition of Stop Solution

Figure 28: Measurement of Absorbance using Microplate Reader

2.7 Preparation of Standard Curve of CXCL4

For calculation of concentration from the obtained absorbance values of CXCL4, a standard curve is required. Standard curve required was obtained by using the known concentrations of the standard 1-7 and their obtained absorbance values. The values used for the preparation of the standard curve are as follows-

Table 6: Required Data for the Preparation of Standard Curve of CXCL4

Concentration (pg/ml)	Absorbance
10000	4.0873
5000	2.7908
2500	1.5477

1250	0.8345
625	0.4675
313	0.3693
156	0.137

The standard curve was plotted by taking the absorbance along the Y-axis and concentrations along the X-axis. The obtained standard curve is presented below.

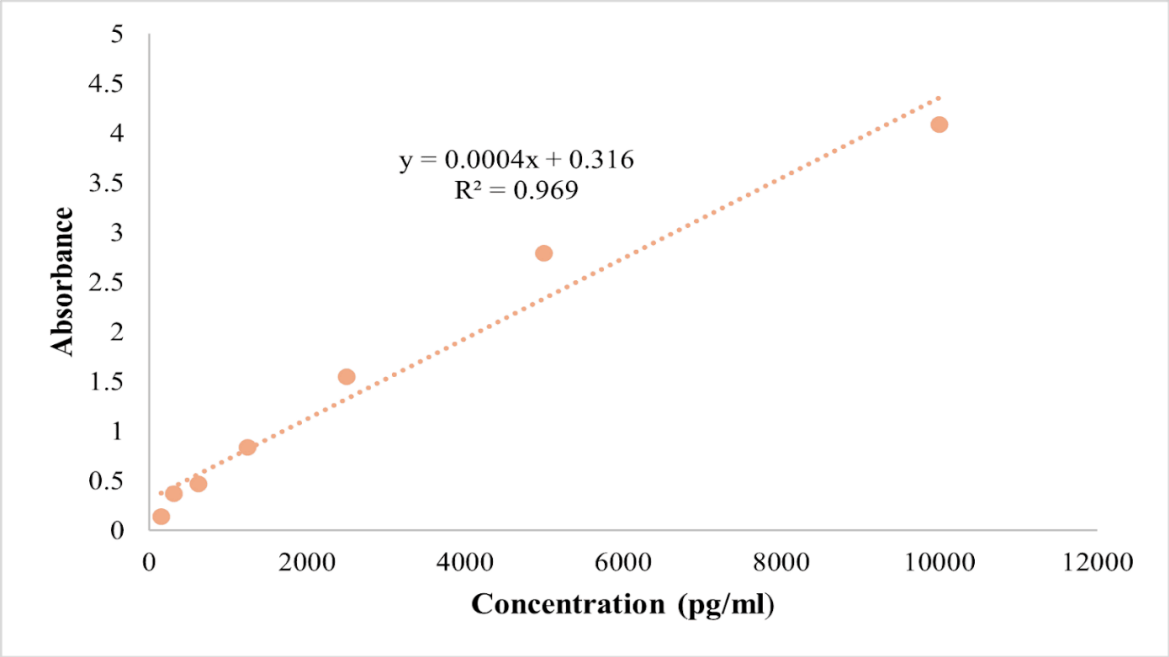


Figure 30: Standard Curve for CXCL4

From the graph, we get the desired straight-line equation, y= 0.0004x+0.316.

2.8 Procedure for Statistical Analysis

For statistical analysis, the Statistical Package for Social Sciences, SPSS software and Microsoft excel was used. Microsoft excel was utilized for data processing. For analysis of all the obtained numerical data, t-test was done to differentiate between the two groups. A Chi-squared (χ^2) test was also conducted to determine the sociodemographic and biophysical characteristic significance between the OCD patients and the HCs, the results were expressed in mean ± SEM after a descriptive statistical calculation. The obtained results were considered statistically significant when the p-value was found to be less than 0.05.

Chapter 3

Results

3.1 Sociodemographic Attributes

The detailed analysis of sociodemographic characteristics of the study population is presented in table 7. The table consists of differential analysis based on several characteristics. The table presents a comparison between the patients (n=200) and HCs (n=176). It contains the % of participants of patients and HCs for each category for a better representation of the differences.

The first differentiating characteristic is the marital status between the patients and controls. Among the controls, the percentage of unmarried participants was 46.59% where the percentage for married individuals was 53.41%. On the other hand, 48% of the patients were married and the rest 52% were unmarried. In both cases, the percentage of unmarried individuals was higher than married. However, the p-value obtained was 0.295 which is >0.05, thus no statistically significant relationship was established.

Similarly, no significant relationship difference was found based on education levels as the p-value obtained was 0.018 which is >0.05. Among the controls, the percentage of graduate level, illiterate, primary level and secondary level participants were found to be 52.84%, 0%, 10.23% and 36.93% respectively. Comparatively, among the patients, 46% participants were in the graduate and above level, 5% were illiterate, 9% had primary level education and 40% had secondary education. No significant difference was observed between the education levels of the two groups.

Based on the occupation, 5.68%, 18.18%, 2.27%, 28.41%, 16.48% and 28.98% of participants among the control were involved in business, were housewife, other occupations, service, students, and unemployed respectively. For patients, documented occupations were business 25%, housewife 18.5%, others 4%, service 29%, student 13% and 33% unemployed. No significant difference was found as the p-value was higher than 0.05, that is 0.493.

Regarding the area of residence, 30.11% of the control participants were from rural areas and 69.89% were from urban areas. And 30% of patients were from rural areas with the rest 70% from urban areas. In this case, again, the p-value was higher than 0.05, that is 0.981. Thus, no significant difference was concluded.

As per economic impression, high impression was observed in 17.05% control and 11% patients. Low impression was found in 31.82% control and 30% patients. Lastly, medium level

impression was found in 51.14% and 59% control and patients respectively. Here the p-value was 0.164 which indicated that there was no statistical significance between this characteristic of the patient and control group.

Again, no significant difference was observed in between the religion of the control and patient groups with a p value of 0.501 which is > 0.05.

Lastly, no significant differences could be found in the smoking history of the patient and control group. In both cases for control and patients, the non-smoker percentage was 82.39% and 82.5% respectively. And the smoker percentage was 17.61% and 17.5% respectively. The obtained p-value was 0.977 that is >0.05. No statistically significant difference was observed.

Table 7: Sociodemographic Attributes of the Study Population

Characteristics	Control (n=176)	%	Patients (n=200)	%	p- value
Marital Status					0.295
Married	94	53.41	96	48	
Unmarried	82	46.59	104	52	
Education level					0.018
Graduate and above	93	52.84	92	46	
Secondary	65	36.93	80	40	
Primary	18	10.23	18	9	
Illiterate	0	0.00	10	5	

Characteristics	Control (n=176)	%	Patients (n=200)	%	p- value
Occupation					0.493
Business	10	5.68	5	2.5	
Housewife	32	18.18	37	18.5	
Others	4	2.27	8	4	
Service	50	28.41	58	29	
Student	29	16.48	26	13	
Unemployed	51	28.98	66	33	
Area of Residence					0.981
Rural	53	30.11	60	30	
Urban	123	69.89	140	70	
Economic Impression					0.164
High	30	17.04	22	11	
Medium	90	51.14	118	59	
Low	56	31.82	60	30	

Characteristics	Control (n=176)	%	Patients (n=200)	%	p- value
Smoking History					0.977
Non-smoker	145	82.39	165	82.5	
Smoker	31	17.61	35	17.5	
Religion					0.501
Islam	169	96.02	188	94	
Hindu	7	3.98	11	5.50	
Buddhist	0	0	1	0.5	

None of the categories showed any significant difference when comparing the OCD patients with the group of HCs, with all categories having a p-value greater than 0.05.

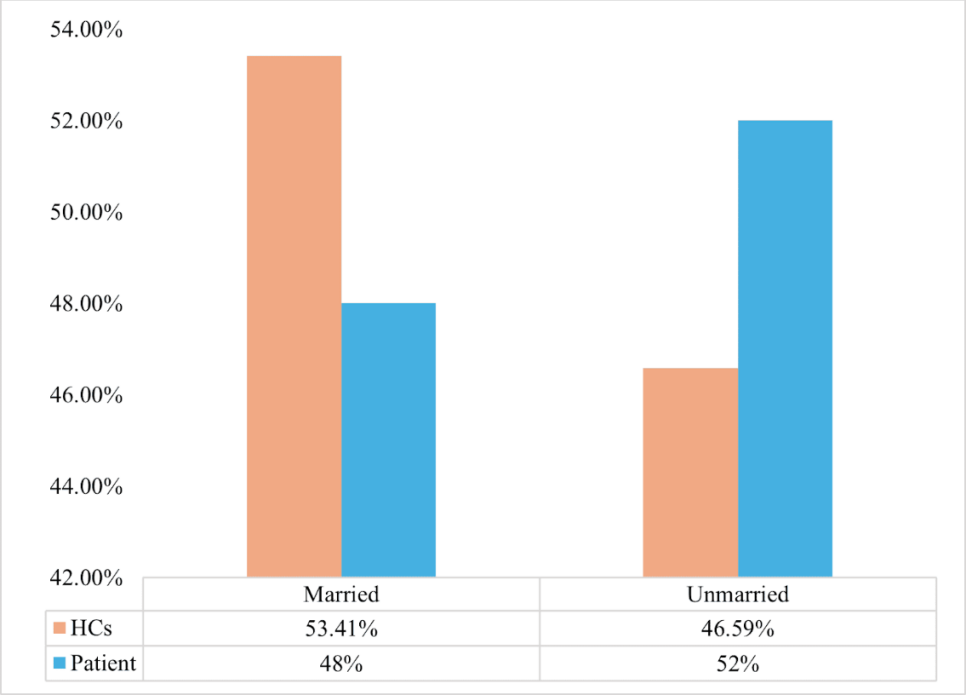
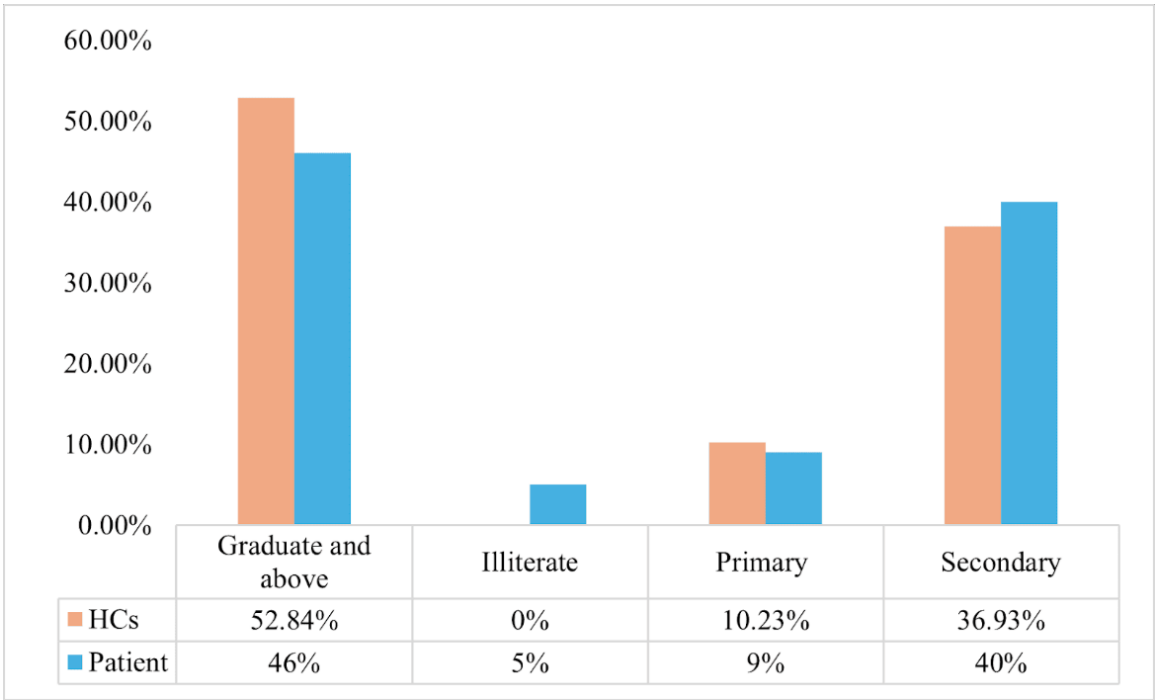
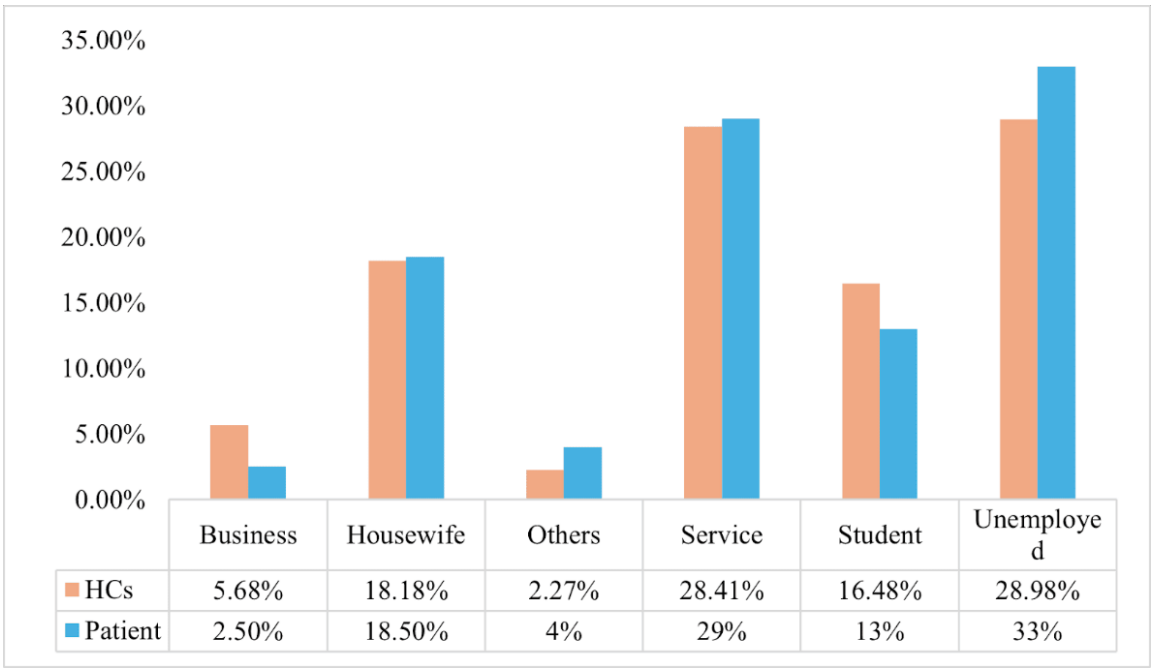


Figure 30: Comparison of Marital Status between Healthy Controls and Patients



	Graduate and above	Illiterate	Primary	Secondary
HCs	52.84%	0%	10.23%	36.93%
Patient	46%	5%	9%	40%

Figure 31: Comparison of Education level between Healthy Controls and patients



	Business	Housewife	Others	Service	Student	Unemployed
HCs	5.68%	18.18%	2.27%	28.41%	16.48%	28.98%
Patient	2.50%	18.50%	4%	29%	13%	33%

Figure 32: Comparison of Occupation between Healthy Controls and OCD patients

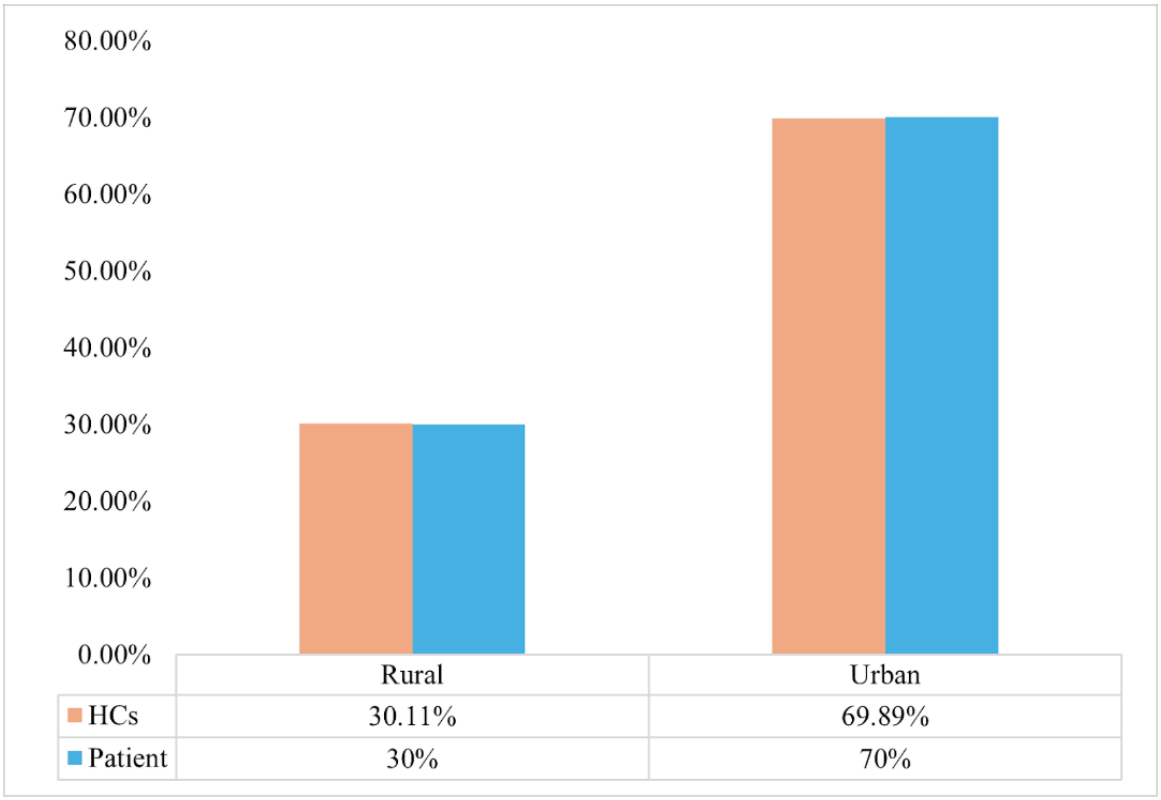


Figure 33: Distribution of Healthy Controls and OCD patients based on Area of Residence

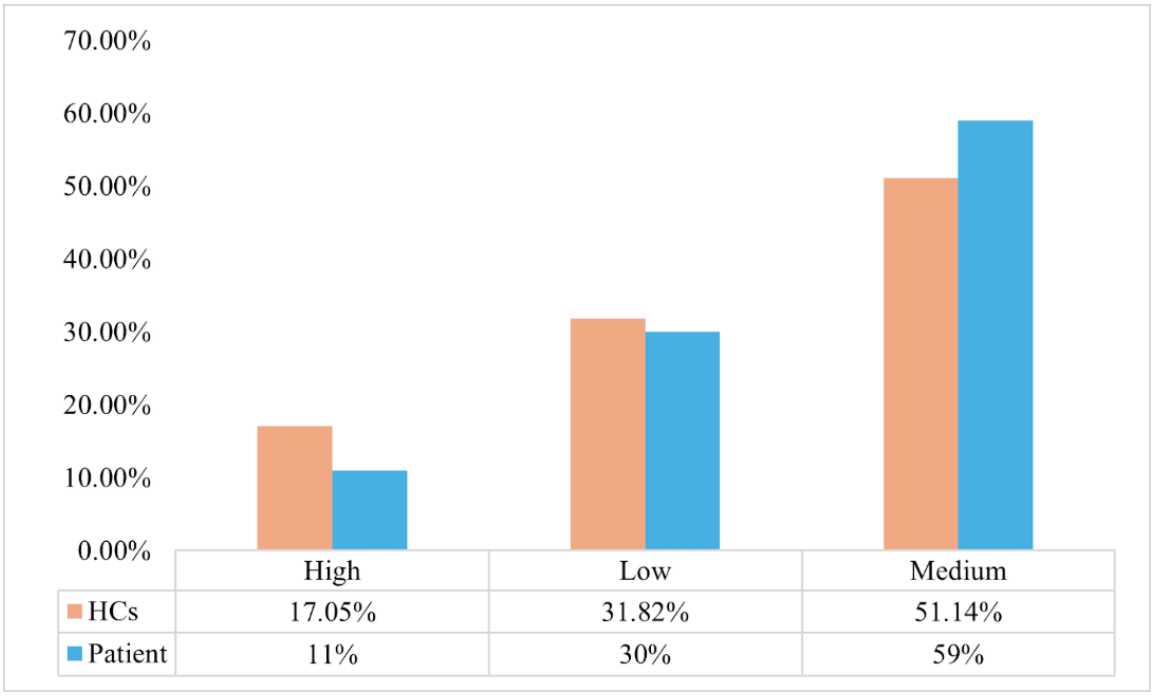


Figure 34: Comparison of Economic Impression between Healthy Controls and OCD patients

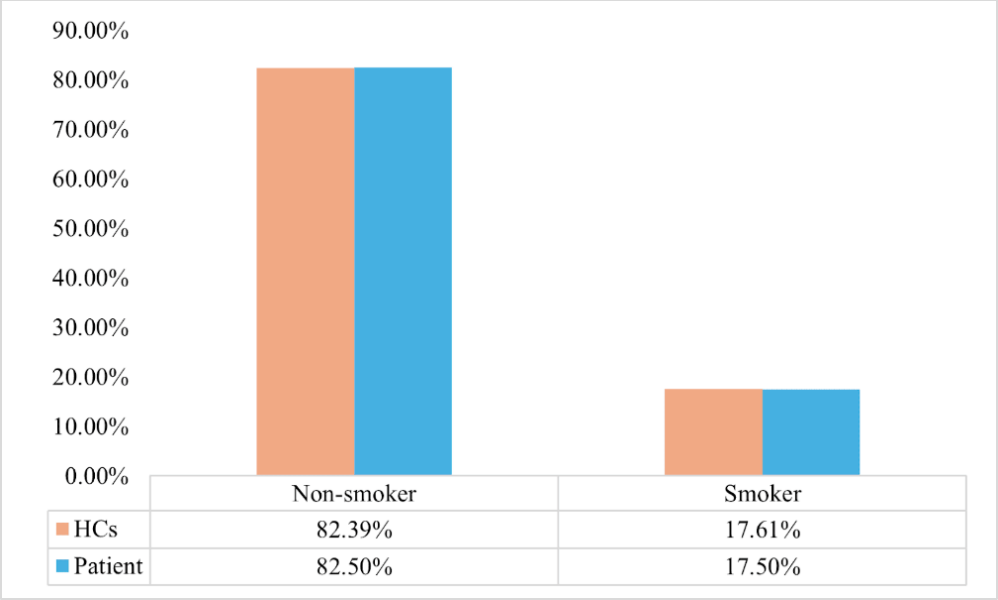


Figure 35: Smoking History Comparison between Healthy Controls and OCD patients

3.2 Biophysical Attributes

The detailed analysis of biophysical characteristics of the study population is presented in table 8. The table consists of differential analysis based on several categories: age, gender and body mass index (BMI). The table presents a comparison between the patients (n=200) and HCs (n=176). It contains the % of participants of patients and controls (HCs) for each category for a better representation of the differences along with the p-values.

Table 8: Biophysical Attributes of the Study Population

Characteristics	Patients (n=200)	%	Control (n=176)	%	p- value
Age					0.161
18-25	102	51	73	41.48	
26-35	54	27	64	36.36	
36-45	32	16	25	14.21	
46-60	12	6	14	7.96	

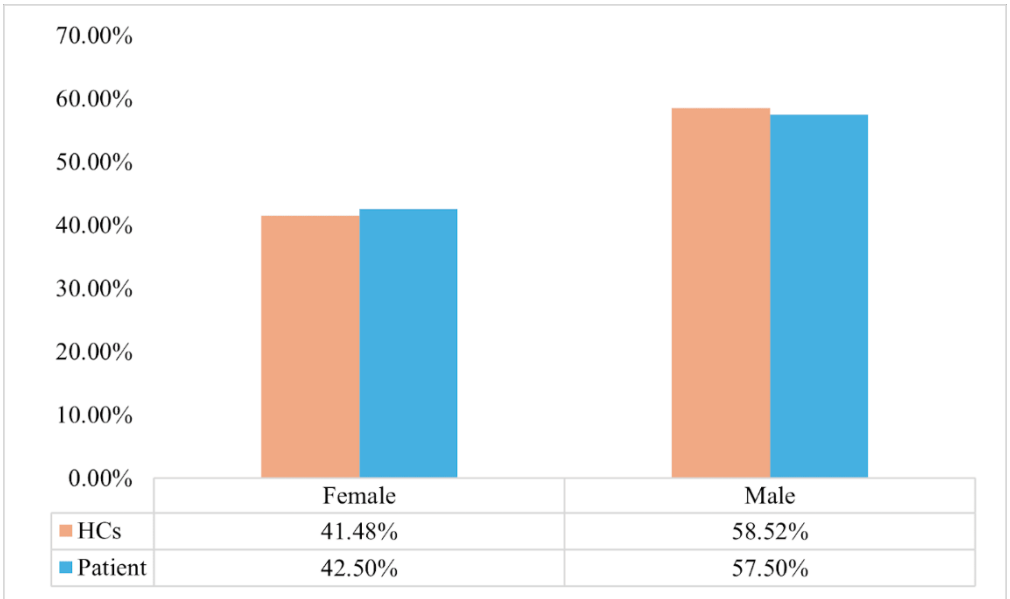
Characteristics	Patients (n=200)	%	Control (n=176)	%	p- value
Gender					0.841
Female	85	42.5	73	41.48	
Male	115	57.5	103	58.52	
BMI (kg/m^2)					<0.001
CED (below 18.5)	28	14	0	0.00	
Normal (18.5-25.0)	130	65	120	68.18	
Overweight (above 25.0)	42	21	56	31.82	

From table 8, Based on the age, among the controls, 41.48%, 36.36%, 14.21 and 7.96% of the participants were from 18-25, 26-35, 36-45 and 46-60 years of age range respectively. Whereas, 51% were between 18-25 years, 27% were 26-35 years, 16% belonged to 36-45 years and 6% of the patients belonged to 46-60 years. Here, the p-value was determined to be 0.161. That is greater than 0.05. Therefore, there is no considerable difference in terms of age between the two groups.

Regarding gender, it was found that 41.48% of the control population were female and 58.52% were male. Comparatively in patients, 42.5% were female with 57.5% male. With a p-value of greater than 0.05, that is 0.841, there was no significant difference between the two groups in the study population based on gender.

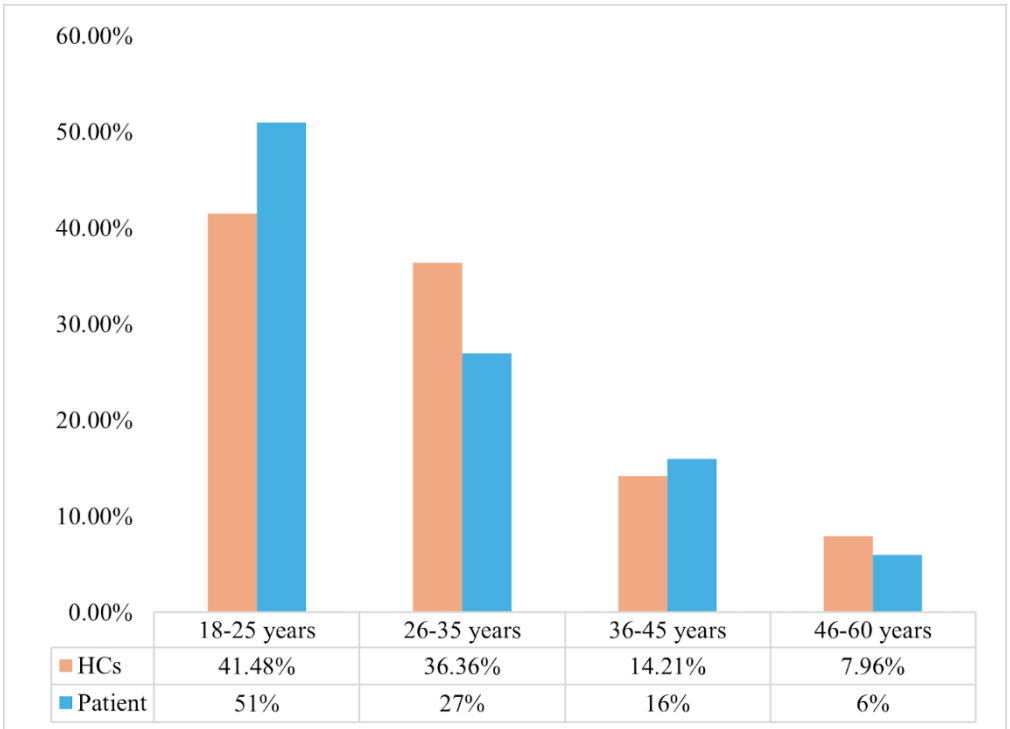
The BMI was categorized into 3 classes, below 18.5 (CED), 18.5-25.0 (Normal) and above 25.0 (Overweight). Among patients, 14% were classified as CED, 65% classified as normal and 21% were classified as overweight. On the other hand, 68.18% were classified as normal,

31.82% were classified as overweight and none was classified as CED among the controls. And the p value was found to be less than 0.001. Here, as the p value is less than 0.05 which indicates a possible statistically significant difference. Before interpreting this statistical significance and the clinical effects, we must consider some other factors too. The considerations for no statistically significant differences based on BMI is further elaborated in the clinical findings section.



	Female	Male
HCs	41.48%	58.52%
Patient	42.50%	57.50%

Figure 36: Comparison of Gender between Healthy Controls and OCD patients



	18-25 years	26-35 years	36-45 years	46-60 years
HCs	41.48%	36.36%	14.21%	7.96%
Patient	51%	27%	16%	6%

Figure 37: Age Distribution between Healthy Controls and OCD patients

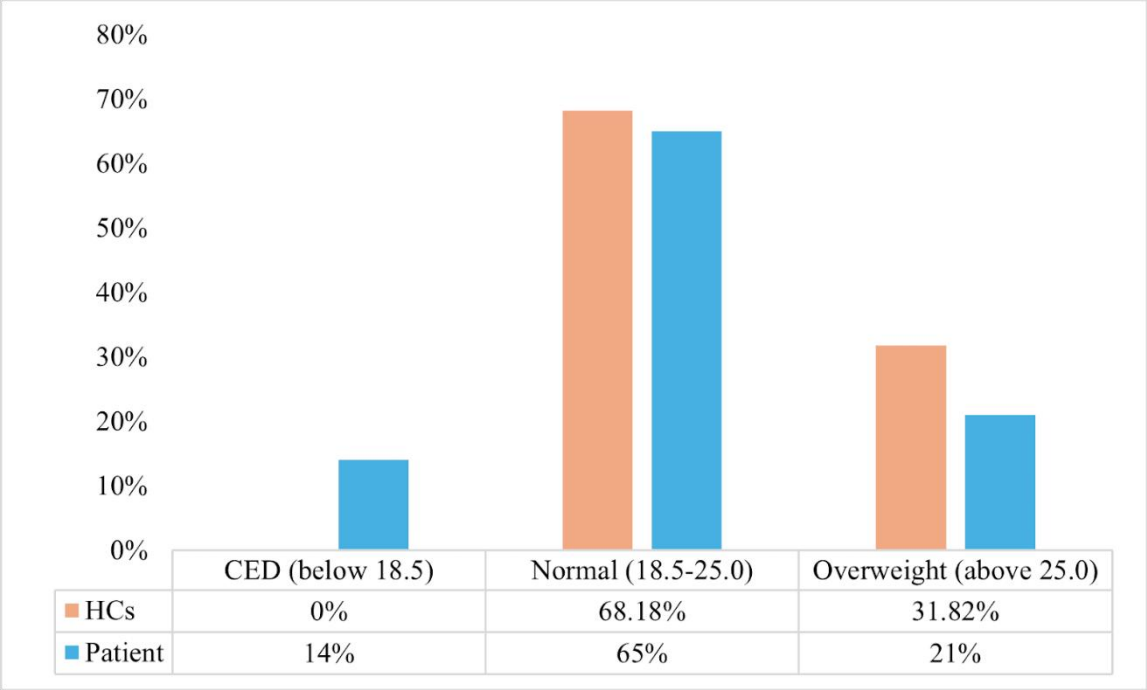


Figure 38: BMI Comparison between Healthy Controls and OCD patients

3.3 Clinical and Analytical Outcomes

The clinical and laboratory findings of the two groups of HCs (n=176) and patients of OCD (n=200) are given in table 9. The table contains the four categories: age, BMI, Y-BOCS score and the concentration of CXCL4 for each group. The total number (N), mean along with Standard Error of the Mean (SEM) and their p-values are demonstrated for each category.

Table 9: Clinical Outcomes and Laboratory Findings

Parameters	Group	N	Mean ± SEM	p-value
Age				0.161
	Patient	200	28.66 ± 0.65	
	Control	176	29.9 ± 0.67	

Parameters	Group	N	Mean ± SEM	p-value
BMI				<0.001
	Patient	200	22.19 ± 0.34	
	Control	176	23.47 ± 0.11	
Y-BOCS Score				<0.001
	Patient	200	22.74± 0.52	
	Control	176	3.44± 0.15	
CXCL4 CONC. (ng/mL)				0.118
	Patient	200	256.48 ± 7.46	
	Control	176	273.39 ± 7.80	

The mean age for control group (n=176) was 29.9 0.67 years and for patients' group (n=200) was 28.66±0.65 with a p value of 0.161. Indicating no possible statistical significance. Conversely, in case of BMI, the score was <0.001, indicating possible statistical significance. For interpretation of any clinically significant difference of BMI between the control and the patient group, we must consider other factors.

Firstly, the normal range for BMI is 18.5-25.0. In this case, the mean BMI for more control and patient groups, that are 23.47 ± 0.11 and 22.19 ± 0.34 respectively both are within the normal range. Therefore, it is highly unlikely for this statistical significance to actually indicate any possible clinical impact of the BMI difference on OCD. As in both cases, the mean is within the acceptable normal range. So, we can suggest that this one p-value cannot conclude the relation between both the groups. Moreover, BMI can be influenced by multiple factors

like diet, age, metabolism, genetics, physical activity etc. To establish any further significance of difference between two groups based on BMI, statistical analysis could be done in more than one test.

The mean Y-BOCS score for HCs (n=176) was found to be 3.44 ± 0.15 while for the patients' group (n=200) it was found to be 22.74 ± 0.52. Here, considering the p value, a statistically significant difference was observed. Here, observing the p value is less than 0.001 with the mean ± SEM, we can conclude that this difference of score serves as a parameter to ascertain that the severity of OCD is much elevated in the patients compared to the controls. According to the Y-BOCS, the mean score for the control group is subclinical whereas the score for the patient group falls within moderate to severe OCD.

Lastly, the mean concentration of CXCL4 (ng/ml) was found to be lower in the patients compared to the HCs. The control group had an average concentration of 273.39 ± 7.80 ng/ml and among the patients it was 256.48 ± 7.46 ng/ml. The obtained p-value was 0.118. This denotes that even though there was a marked reduction in CXCL4 levels, the difference was not statistically significant.

Chapter 4

Discussion

Among the sociodemographic categories analyzed for the two groups of patients and HCs, no significant differences were found based on the marital status, area of residence, occupation, smoking history, economic impression and education level in this specific study population. Moreover, in the case of biophysical parameters, of age or gender, this study did not indicate any statistically significant differences within the two groups analyzed within this study population. These statistical findings signifies that the features were evenly distributed amongst the two groups, which reduces the possibility of such variables causing any potential impact on the final outcome of the research study. Again, the Y-BOCS score among the clinical findings indicates that the score was significantly higher in case of the patients compared to that of the HCs. This also emphasizes that the accuracy and recruitment of participants in the study population was suitable for providing an accurate interpretation of the results.

This study explored the serum level of CXCL4 between two groups, one composed of HCs (n=176) and another composed of clinically diagnosed OCD patients (n=200). According to the assay, and after extensive statistical analysis, the resulting concentrations of CXCL4 were found to be slightly lower among the OCD patient serum than that of the HC individuals. Even though CXCL4 is a strongly proinflammatory cytokine with major roles in inflammation including receptor independent immune cell recruitment.

Regardless of the current view of possible effects of immune dysregulation on the pathophysiology of OCD, the results of analysis involving inflammatory markers remain inconsistent (Cosco et al., 2019). The insignificance of CXCL4 aligns with multiple meta-analyses conducted on various proinflammatory biomarkers where no statistical significance could be established between the serum level of the markers and OCD patients compared to that of HCs. Some such proinflammatory markers are Interleukin-1 (IL-1), tumor necrosis factor- α (TNF-α), interferon-γ, Interleukin-1β (IL-1β), Interleukin-6 (IL-6) and Interleukin-4 (IL-4), with which no significant difference was observed between OCD patients and controls (Cosco et al., 2019).

On the other hand, the difference was found to be statistically insignificant due to a p-value greater than 0.05 which is 0.118. The findings of this study may suggest that CXCL4 does not have any clear association with the pathophysiology of OCD, even though CXCL4 is a strongly

proinflammatory cytokine. However, the slight reduction of CXCL4 levels in patients compared to the control along with the p-value of no statistical significance, suggests that there is no clear correlation between the decrease of CXCL4 in OCD, but it also does not confirm any anti-inflammatory effect on OCD. In another contradicting study, there was a statistically significant decrease in proinflammatory marker IL-1β levels in OCD patients compared to control observed, suggesting a possible anti-inflammatory effect (Gray & Bloch, 2012).

This study design has a few limitations. As the study involved a single biomarker, is it not sufficient to explore the vast multifactorial pathophysiology of OCD. More comprehensive data collection such as- involving serum concentration correlating with Y-BOCS severity along with gender specific parameters could have provided a newer aspect to the obtained result. Also, a cohort study involving one anti and pro-inflammatory chemokine could have strengthened the basis of the project.

Despite its limitations, the study does have some advantages. One of the most notable advantages is that this is the first case-control study evaluating the concentration of proinflammatory cytokine CXCL4 in serum levels of OCD patients and HCs. Moreover, the large and sociodemographically diverse sample size of the study has increased the dependability and generalizability of the study. This demonstrated that the study has a reliable accuracy in depicting the demographic distribution within this geographic area.

Therefore, this study concludes that the concentration of serum CXCL4 level has no statistical significance among this study population. As there has been no analysis of serum levels of CXCL4 till now, this single study is not significant enough to ascertain whether it has a marked impact on pathophysiology of OCD or not. Further research involving intervention and multi geographical study design is required to establish the activity of this biomarker in the pathophysiology of OCD. Moreover, combining CXCL4 with another pro-inflammatory or anti-inflammatory biomarker could assist in increasing the specificity of the study and validate the possible effects of immune dysfunction in OCD pathophysiology.

Chapter 5

Conclusion

This study focuses on identifying any possible association between serum CXCL4 level among healthy and individuals with OCD to detect whether it can be proposed as a reliable biomarker having any underlying mechanisms contributing to the pathophysiology of OCD. It aimed to contribute to the development of establishing a diagnostic and supporting tool in diagnosis of OCD. Further research is required to validate the key findings of this study and aid in establishing the possible roles of CXCL4 in pathophysiology of OCD.

References

Algin, S., Sajib, M. W. H., & Arafat, S. M. Y. (2018). Phenomenology of obsessive compulsive disorder in Bangladesh: A cross-sectional observation. *Asian Journal of Psychiatry*, *34*, 18–20. https://doi.org/10.1016/j.ajp.2018.04.011

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). American Psychiatric Publishing.

Barlow, D. H. (2014). *Clinical handbook of psychological disorders : a step-by-step treatment manual*. The Guilford Press.

Bhatt, S., Anitha, K., Chellappan, D. K., Mukherjee, D., Shilpi, S., Suttee, A., Gupta, G., Singh, T. G., & Dua, K. (2024). Targeting inflammatory signaling in obsessive compulsive disorder: a promising approach. *Metabolic brain disease*, *39*(2), 335–346. https://doi.org/10.1007/s11011-023-01314-3

Bora, E. (2022). Social cognition and empathy in adults with obsessive compulsive disorder: A meta-analysis. *Psychiatry Research*, *316*, 114752. https://doi.org/10.1016/j.psychres.2022.114752

Brakoulias, V., Starcevic, V., Belloch, A., Brown, C., Ferrao, Y. A., Fontenelle, L. F., Lochner, C., Marazziti, D., Matsunaga, H., Miguel, E. C., Reddy, Y. C. J., do Rosario, M. C., Shavitt, R. G., Shyam Sundar, A., Stein, D. J., Torres, A. R., & Viswasam, K. (2017). Comorbidity, age of onset and suicidality in obsessive–compulsive disorder (OCD): An international collaboration. *Comprehensive Psychiatry*, *76*, 79–86. https://doi.org/10.1016/j.comppsych.2017.04.002

Castro-Rodrigues, P., Camacho, M., Almeida, S., Marinho, M., Soares, C., Barahona-Corrêa, J. B., & Oliveira-Maia, A. J. (2018). Criterion Validity of the Yale-Brown Obsessive-Compulsive Scale Second Edition for Diagnosis of Obsessive-Compulsive Disorder in Adults. *Frontiers in Psychiatry*, *9*(431). https://doi.org/10.3389/fpsyt.2018.00431

Cervin, M. (2023). Obsessive-Compulsive Disorder. *Psychiatric Clinics of North America*, *46*(1), 1–16. https://doi.org/10.1016/j.psc.2022.10.006

Chowdhury, A. D. , Hossain, M. D., Haq, A. I., Kowser, A. S. M., Khan, M. S. A., & Parveen, M. (2020). Psychiatric comorbidity in patients with obsessive-compulsive disorder. Archives of NIMH, 3, 16–20. https://nimh.gov.bd/english/wp-

content/uploads/2023/11/04-Original-Article-Psychiatric-comorbidity-in-patients-with-obsessive-compulsive-disorder.pdf

Cosco, T. D., Pillinger, T., Emam, H., Solmi, M., Budhdeo, S., Matthew Prina, A., Maes, M., Stein, D. J., Stubbs, B., & Carvalho, A. F. (2019). Immune Aberrations in Obsessive-Compulsive Disorder: a Systematic Review and Meta-analysis. *Molecular neurobiology*, *56*(7), 4751–4759. https://doi.org/10.1007/s12035-018-1409-x

Del Casale, A., Sorice, S., Padovano, A., Simmaco, M., Ferracuti, S., Lamis, D. A., Rapinesi, C., Sani, G., Girardi, P., Kotzalidis, G. D., & Pompili, M. (2019). Psychopharmacological Treatment of Obsessive-Compulsive Disorder (OCD). *Current Neuropharmacology*, *17*(8), 710–736. https://doi.org/10.2174/1570159x16666180813155017

Geller, D., Biederman, J., Jones, J., Park, K., Schwartz, S., Shapiro, S., & Coffey, B. (1998). Is Juvenile Obsessive-Compulsive Disorder a Developmental Subtype of the Disorder? A Review of the Pediatric Literature. *Journal of the American Academy of Child & Adolescent Psychiatry*, *37*(4), 420–427. https://doi.org/10.1097/00004583-199804000-00020

Goodman, W. K., Grice, D. E., Lapidus, K. A. B., & Coffey, B. J. (2014). Obsessive-compulsive disorder. *The Psychiatric Clinics of North America*, *37*(3), 257–267. https://doi.org/10.1016/j.psc.2014.06.004

Goodman, W. K., Price, L. H., Rasmussen, S. A., Mazure, C., Fleischmann, R. L., Hill, C. L., Heninger, G. R., & Charney, D. S. (1989). The Yale-Brown Obsessive Compulsive Scale. I. Development, use, and reliability. *Archives of General Psychiatry*, *46*(11), 1006–1011. https://doi.org/10.1001/archpsyc.1989.01810110048007

Goodman, W. K., Storch, E. A., & Sheth, S. A. (2021). Harmonizing the Neurobiology and Treatment of Obsessive-Compulsive Disorder. *American Journal of Psychiatry*, *178*(1), 17–29. https://doi.org/10.1176/appi.ajp.2020.20111601

Gray, S. M., & Bloch, M. H. (2012). Systematic review of proinflammatory cytokines in obsessive-compulsive disorder. *Current psychiatry reports*, *14*(3), 220–228. https://doi.org/10.1007/s11920-012-0272-0

Gray, A. L., Karlsson, R., Roberts, A. R. E., Ridley, A. J. L., Pun, N., Khan, B., Lawless, C., Luís, R., Szpakowska, M., Chevigné, A., Hughes, C. E., Medina-Ruiz, L., Birchenough,

H. L., Mulholland, I. Z., Salanga, C. L., Yates, E. A., Turnbull, J. E., Handel, T. M., Graham, G. J., & Jowitt, T. A. (2023). Chemokine CXCL4 interactions with extracellular matrix proteoglycans mediate widespread immune cell recruitment independent of chemokine receptors. *Cell Reports*, *42*(1), 111930. https://doi.org/10.1016/j.celrep.2022.111930

Hollander, E., Stein, D. J., Fineberg, N. A., Marteau, F., & Legault, M. (2010). Quality of Life Outcomes in Patients With Obsessive-Compulsive Disorder. *The Journal of Clinical Psychiatry*, *71*(06), 784–792. https://doi.org/10.4088/jcp.09m05911blu

Hollander, E., Stein, D. J., Kwon, J. H., Rowland, C., Wong, C. M., Broatch, J., & Himelein, C. (1998). Psychosocial Function and Economic Costs of Obsessive-Compulsive Disorder. *CNS Spectrums*, *3*(S1), 48–58. https://doi.org/10.1017/s1092852900007239

Hozhabri, H., Moghaddam, M. M., Moghaddam, M. M., & Mohammadian, A. (2022). A comprehensive bioinformatics analysis to identify potential prognostic biomarkers among CC and CXC chemokines in breast cancer. *Scientific reports*, *12*(1), 10374. https://doi.org/10.1038/s41598-022-14610-2

Hughes, C. E., & Nibbs, R. J. B. (2018). A guide to chemokines and their receptors. *The Febs Journal*, *285*(16), 2944–2971. https://doi.org/10.1111/febs.14466

Ji, Y., Zhang, Q., Li, H., Chen, L., Wu, Y., & Lin, S. (2025). Platelet Factor 4: A Mysterious Chemokine in Inflammatory Regulation Diseases. *Journal of Inflammation Research*, *Volume 18*, 4481–4495. https://doi.org/10.2147/jir.s504673

Kaczor, D. M., Kramann, R., Hackeng, T. M., Schurgers, L. J., & Koenen, R. R. (2022). Differential Effects of Platelet Factor 4 (CXCL4) and Its Non-Allelic Variant (CXCL4L1) on Cultured Human Vascular Smooth Muscle Cells. *International Journal of Molecular Sciences*, *23*(2), 580. https://doi.org/10.3390/ijms23020580

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime Prevalence and age-of-onset Distributions of DSM-IV Disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry*, *62*(6), 593–602. https://doi.org/10.1001/archpsyc.62.6.593

Kurki, M. I., Karjalainen, J., Palta, P., Sipilä, T. P., Kristiansson, K., Donner, K. M., Reeve, M. P., Laivuori, H., Aavikko, M., Kaunisto, M. A., Loukola, A., Lahtela, E., Mattsson, H., Laiho, P., Della Briotta Parolo, P., Lehisto, A. A., Kanai, M., Mars, N., Rämö, J.,

& Kiiskinen, T. (2023). FinnGen provides genetic insights from a well-phenotyped isolated population. *Nature*, *613*(7944), 508–518. https://doi.org/10.1038/s41586-022-05473-8

Lochner, C., Maré, K. T., & Stein, D. J. (2024). Structured clinical interview for diagnosing obsessive-compulsive spectrum disorders. *Comprehensive psychiatry*, *133*, 152494. https://doi.org/10.1016/j.comppsych.2024.152494

Manne, B. K., Xiang, S. C., & Rondina, M. T. (2017). Platelet secretion in inflammatory and infectious diseases. *Platelets,* *28*(2), 155–164. https://doi.org/10.1080/09537104.2016.1240766

Pampaloni, I., Marriott, S., Pessina, E., Fisher, C., Govender, A., Mohamed, H., Chandler, A., Tyagi, H., Morris, L., & Pallanti, S. (2022). The global assessment of OCD. *Comprehensive Psychiatry*, *118*(152342), 152342. https://doi.org/10.1016/j.comppsych.2022.152342

Piacentini, J., Bergman, R. L., Keller, M., & McCracken, J. (2003). Functional Impairment in Children and Adolescents with Obsessive-Compulsive Disorder. *Journal of Child and Adolescent Psychopharmacology*, *13*(supplement 1), 61–69. https://doi.org/10.1089/104454603322126359

Pittenger, C. (2021). Pharmacotherapeutic Strategies and New Targets in OCD. *The Neurobiology and Treatment of OCD: Accelerating Progress*, *49*, 331–384. https://doi.org/10.1007/7854_2020_204

Rachman, S. (2012). Health anxiety disorders: A cognitive construal. *Behaviour Research and Therapy*, *50*(7-8), 502–512. https://doi.org/10.1016/j.brat.2012.05.001

Reid, J. E., Laws, K. R., Drummond, L., Vismara, M., Grancini, B., Mpavaenda, D., & Fineberg, N. A. (2021). Cognitive behavioural therapy with exposure and response prevention in the treatment of obsessive-compulsive disorder: A systematic review and meta-analysis of randomised controlled trials. *Comprehensive Psychiatry*, *106*(152223), 152223. https://doi.org/10.1016/j.comppsych.2021.152223

Rodríguez, N., Morer, A., González-Navarro, E. A., Serra-Pages, C., Boloc, D., Torres, T., García-Cerro, S., Mas, S., Gassó, P., & Lázaro, L. (2017). Inflammatory dysregulation of monocytes in pediatric patients with obsessive-compulsive disorder. *Journal of neuroinflammation*, *14*(1), 261. https://doi.org/10.1186/s12974-017-1042-z

Shi, G., Field, D. J., Ko, K. A., Ture, S., Srivastava, K., Levy, S., Kowalska, M. A., Poncz, M., Fowell, D. J., & Morrell, C. N. (2014). Platelet factor 4 limits Th17 differentiation and cardiac allograft rejection. *The Journal of clinical investigation*, *124*(2), 543–552. https://doi.org/10.1172/JCI71858

Singh, A., Anjankar, V., & Sapkale, B. (2023). Obsessive-Compulsive Disorder (OCD): A Comprehensive Review of Diagnosis, Comorbidities, and Treatment Approaches. *Cureus*, *15*(11). https://doi.org/10.7759/cureus.48960

Sinopoli, V. M., Burton, C. L., Kronenberg, S., & Arnold, P. D. (2017). A review of the role of serotonin system genes in obsessive-compulsive disorder. *Neuroscience & Biobehavioral Reviews*, *80*, 372–381. https://doi.org/10.1016/j.neubiorev.2017.05.029

Skapinakis, P., Caldwell, D. M., Hollingworth, W., Bryden, P., Fineberg, N. A., Salkovskis, P., Welton, N. J., Baxter, H., Kessler, D., Churchill, R., & Lewis, G. (2021). Pharmacological and Psychotherapeutic Interventions for Management of Obsessive-compulsive Disorder in Adults: A Systematic Review and Network Meta-analysis. *Focus (American Psychiatric Publishing)*, *19*(4), 457–467. https://doi.org/10.1176/appi.focus.19402

Solmi, M., Radua, J., Olivola, M., Croce, E., Soardo, L., Salazar de Pablo, G., Il Shin, J., Kirkbride, J. B., Jones, P., Kim, J. H., Kim, J. Y., Carvalho, A. F., Seeman, M. V., Correll, C. U., & Fusar-Poli, P. (2021). Age at onset of mental disorders worldwide: large-scale meta-analysis of 192 epidemiological studies. *Molecular Psychiatry*, *27*(1), 281–295. https://doi.org/10.1038/s41380-021-01161-7

Stein, D. J., Kogan, C. S., Atmaca, M., Fineberg, N. A., Fontenelle, L. F., Grant, J. E., Matsunaga, H., Reddy, Y. C. J., Simpson, H. B., Thomsen, P. H., van den Heuvel, O. A., Veale, D., Woods, D. W., & Reed, G. M. (2016). The classification of Obsessive–Compulsive and Related Disorders in the ICD-11. *Journal of Affective Disorders*, *190*, 663–674. https://doi.org/10.1016/j.jad.2015.10.061

Storch, E. A., Larson, M. J., Price, L. H., Rasmussen, S. A., Murphy, T. K., & Goodman, W. K. (2010). Psychometric analysis of the Yale-Brown Obsessive–Compulsive Scale Second Edition Symptom Checklist. *Journal of Anxiety Disorders*, *24*(6), 650–656. https://doi.org/10.1016/j.janxdis.2010.04.010

Storch, E. A., Rasmussen, S. A., Price, L. H., Larson, M. J., Murphy, T. K., & Goodman, W. K. (2010). Development and psychometric evaluation of the Yale–Brown Obsessive-

Compulsive Scale—Second Edition. *Psychological Assessment*, *22*(2), 223–232. https://doi.org/10.1037/a0018492

van Bon, L., Affandi, A. J., Broen, J., Christmann, R. B., Marijnissen, R. J., Stawski, L., Farina, G. A., Stifano, G., Mathes, A. L., Cossu, M., York, M., Collins, C., Wenink, M., Huijbens, R., Hesselstrand, R., Saxne, T., DiMarzio, M., Wuttge, D., Agarwal, S. K., Reveille, J. D., … Radstake, T. R. (2014). Proteome-wide analysis and CXCL4 as a biomarker in systemic sclerosis. *The New England journal of medicine*, *370*(5), 433–443. https://doi.org/10.1056/NEJMoa1114576

Wang, Z., & Huang, H. (2013). Platelet factor-4 (CXCL4/PF-4): An angiostatic chemokine for cancer therapy. *Cancer Letters*, *331*(2), 147–153. https://doi.org/10.1016/j.canlet.2013.01.006

Wang, D., Zhang, Z., Cui, S., Zhao, Y., Craft, S., Fikrig, E., & You, F. (2019). ELF4 facilitates innate host defenses against *Plasmodium* by activating transcription of *Pf4* and *Ppbp*. *The Journal of biological chemistry*, *294*(19), 7787–7796. https://doi.org/10.1074/jbc.RA118.006321

Yadav, S., & Storrie, B. (2017). The cellular basis of platelet secretion: Emerging structure/function relationships. *Platelets,* *28*(2), 108–118. https://doi.org/10.1080/09537104.2016.1257786

Yang, C., Bachu, M., Du, Y., Brauner, C., Yuan, R., Ah Kioon, M. D., Chesi, G., Barrat, F. J., & Ivashkiv, L. B. (2022). Costimulation of TLR8 responses by CXCL4 in Human Monocytes Mediated by TBK1-IRF5 Signaling and Epigenomic Remodeling. *The Journal of Immunology*, *208*(Issue supplemeny_1). https://doi.org/10.1101/2022.03.28.486089

Zhang, X., Chen, L., Bancroft, D. P., Lai, C.-S., & Maione, T. E. (1994). Crystal Structure of Recombinant Human Platelet Factor 4. *Biochemistry*, *33*(27), 8361–8366. https://doi.org/10.1021/bi00193a025

Zhou, C., Gao, Y., Ding, P., Wu, T., & Ji, G. (2023). The role of CXCL family members in different diseases. *Cell Death Discovery*, *9*(1). https://doi.org/10.1038/s41420-023-01524-9

Appendix

Appendix A

(Consent form in Bangla)

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

_____ _____

(Consent form in English)

Consent Form

1. Have you fully understood the nature, purpose, and methodology of this research work?	**Yes / No**
2. Have you been informed that blood will be collected by nurses in this research?	**Yes / No**
3. Is your understanding regarding the outcome and potential benefits of this research clear?	**Yes / No**
4. Have you been able to independently make the decision to participate, cooperate, or refrain from this research work?	**Yes / No**
5. Are your fundamental human rights being violated as a result of participating in the research work?	**Yes / No**
6. Are you fully aware that the confidentiality of your information will be maintained?	**Yes / No**
7. Are you adequately informed that you will not be given any remuneration or travel allowance for participating in the research work?	**Yes / No**

Authorization Letter

Having gained a full understanding of the purpose, methodology, and utility of this research work, I express my consent to its ethical principles. I have not been influenced by any individual or group to participate in this research work, nor have my fundamental human rights been violated. Therefore, subject to proper review, I am voluntarily signing this authorization letter.

Name: _____ **Signature:** _____

Appendix B

Questionnaires Form

Study of BDNF, IL-1 Beta, and ApoE in Obsessive-Compulsive Disorder Patients among Bangladeshi Population

1. Identification

1.1 ID Code:

1.2 Name

1.3 Father's/Husband's Name:

1.4 Sex

Male		Female	

1.5 Marital Status

1.6 Date of Birth(dd/mm/yy)

1.7 Age(Yr)

1.8 Phone No

1.9 Permanent Address

1.10 Religion

1.11 Nationality

2. Personal History

2.1	Area of residence	Rural	Urban	S-Urban	Others

1	Where have you spent your Childhood(1-15y)?				
2	Where have you spent at least 3/4th more of your lifetime?				

2.2	**Education Level**		2.3	**Occupation**		
	Illiterate			Professional/Managerial/Business/		
	Can read only			Clerical		
	Can write a letter			Technical		
	SSC or equivalent			Skilled Worker		
	HSC or equivqlent			Unemployed/Pensioner		
	Graduate or Higher			Housewife		
	Others			Others		

2.4	**Impression about Social Class**		2.4	**Family Expenses/Month**
	Rich			
	Upper Middle			
	Lower Middle			
	Poor			
	Destitute			

2.6	**Smoking Habit**		2.7a	**Current Smoker**	
	Never			Sticks/Day	
	Ex-smoker>6months		2.7b	**Ex-smoker**	
	Current smoker			Sticks/Day	

3. Previous History of Psychiatric Disorder Y N

4. Family History of Psychiatric Disorder: Y N

5. Case: New Old

6. Biological Characteristics:

6.1 Height(cm):

6.2 Weight(kg):

6.3 Pulse/min:

6.4 Temperature:

6.5 BP(sys/dias):

Appendix C

YALE-BROWN OBSESSIVE COMPULSIVE SCALE (Y-BOCS)

Questions 1 to 5 for obsessive thoughts

1. How much of your time is occupied by obsessive thoughts?
   0 = None
   1 = Less than 1 hr/day
   2 = 1 to 3 hrs/day
   3 = Greater than 3 and up to 8 hrs/day
   4 = Greater than 8 hrs/day
2. How much do your obsessive thoughts interfere with your work, school, social or other important role functioning?
   0 = None
   1 = Slight interference, but overall performance not impaired
   2 = Definite interference, but still manageable
   3 = Substantial interference
   4 = Incapacitating
3. How much distress do your obsessive thoughts cause you?
   0 = None
   1 = Not too disturbing
   2 = Disturbing, but still manageable
   3 = Very disturbing
   4 = Near constant and disabling distress
4. How much of an effort do you make to resist the obsessive thoughts?
   0 = Try to resist all the time
   1 = Try to resist most of the time
   2 = Make some effort to resist
   3 = Yield to all obsessions without attempting to control them
   4 = Completely and willingly yield to all obsessions
5. How much control do you have over your obsessive thoughts?
   0 = Try to resist all the time
   1 = Try to resist most of the time
   2 = Make some effort to resist
   3 = Yield to all obsessions without attempting to control them
   4 = Completely and willingly yield to all obsessions

Questions 6 to 10 for compulsive behaviors

6. How much time do you spend performing compulsive behaviors?
   0 = None
   1 = Less than 1 hr/day
   2 = 1 to 3 hrs/day
   3 = Greater than 3 and up to 8 hrs/day
   4 = Greater than 8 hrs/day
7. How much do your compulsive behaviors interfere with your work, school, social or other important role functioning?
   0 = None
   1 = Slight interference, but overall performance not impaired
   2 = Definite interference, but still manageable
   3 = Substantial interference
   4 = Incapacitating
8. How would you feel if prevented from performing your compulsion?
   0 = None
   1 = Only slightly anxious if compulsions prevented
   2 = Anxiety would mount, but remain manageable if compulsions were prevented
   3 = Prominent and very disturbing
   4 = Incapacitating anxiety
9. How much of an effort do you make to resist the compulsions?
   0 = Try to resist all the time
   1 = Try to resist most of the time
   2 = Make some effort to resist
   3 = Yield to all obsessions without attempting to control them
   4 = Completely and willingly yield to all obsessions
10. How strong is the drive to perform the compulsive behavior?
   0 = Complete control
   1 = pressure to perform the behavior
   2 = Make some effort to resist
   3 = Very strong drive to perform behavior
   4 = Drive to perform behavior experienced as completely involuntary

19% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

Filtered from the Report

- Bibliography
- Quoted Text

Match Groups

- 200 Not Cited or Quoted 14%
  Matches with neither in-text citation nor quotation marks
- 76 Missing Quotations 5%
  Matches that are still very similar to source material
- 0 Missing Citation 0%
  Matches that have quotation marks, but no in-text citation
- 0 Cited and Quoted 0%
  Matches with in-text citation present, but no quotation marks

Top Sources

11% Internet sources
10% Publications
15% Submitted works (Student Papers)

Integrity Flags

0 Integrity Flags for Review

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.