Chapter One

Introduction

1.1 Background to the Study

In 2023, the Neuropsychiatric Hospital in Lagos, Nigeria, reported a 100 percent increase in the number of impatient admissions, with depression cases going up by about 43 percent[1]. Out of the 25,000 genes which constitute the human genome, 3 million microbial genes are expressed in every human's gut, these genes are in charge of a variety of metabolic processes, one of which is 95% of the body's serotonin production (both a neurotransmitter and a hormone)[2]. Anxiety and depression have been linked to serotonin deficit, which is necessary for mood, restful sleep, digestion, and bone health[3, 4].

Approximately 3.8% of the population is thought to be impacted, of which 5.0% of adults and 5.7% of individuals over 60 are impacted. Around 280 million people worldwide experience depression[5]. As the number of people who experience depression increased by 18.4% between 2005 and 2015, there is evidence that the prevalence of depression is rising[6]. Every year, depression affects 1 in 15 adults (6.7%). Findings from the Global Burden of Disease (GBD) studies, major depressive disorder (MDD) is recognized as one of the leading contributors to years lived with disability (YLDs) on a global scale[7]. The 2017 GBD estimates indicate that depressive disorders, encompassing both MDD and dysthymia, constituted the third highest cause of YLDs worldwide, representing around 5% of the total burden[7], while suicide is the fourth most common cause of death for people aged 15 to 29 globally[8].

Distinguished by symptoms of sadness, irritability, emptiness, or a loss of pleasure or interest in activities for the majority of the day, nearly every day, for at least two weeks,

poor concentration, excessive guilt or low self-worth, hopelessness about the future, suicidal thoughts, disturbed sleep, changes in appetite and weight, mood changes more readily in the form of physical symptoms (such as pain, fatigue, or weakness), and even depressive symptoms (suicide)[9]. Major Depressive Disorder (MDD) is an extremely serious illness that impairs every aspect of the body's operation.

SLC6A4 gene encodes the serotonin transporter (5-HTT), a significant protein that controls serotonergic neurotransmission, esentially facilitating the reuptake of serotonin from the synaptic cleft (the space between neurons) back into the presynaptic neuron following its release[10]. This reuptake mechanism is vital for terminating serotonergic signaling and maintaining neurotransmitter balance. and has an impact on mood and stress responses. The human serotonin transporter gene, or SLC6A4 (also known as HTT, 5HTT, SERT, 5-HTT, SERT1, or 5-HTTLPR), is located on chromosome 17q11.1–17q12[10] (17q: the long arm of chromosome 17). Several polymorphisms have been found within the SLC6A4 gene. The serotonergic transporter-linked polymorphic region (5-HTTLPR), which is a 44-bp insertion or deletion polymorphism that results in two common alleles, long (L) and short (S), is one of the most commonly investigated functional polymorphisms of the SLC6A4 gene[11]. Increased serotonin binding and re-uptake have been connected to MDD, and the S allele is associated with decreased transcription when it is carried homozygously or heterozygously[11].

Among the medications used to treat depression is the selective serotonin re-uptake inhibitor (SSRI), which blocks the re-uptake of the neurotransmitter and increases its availability[12]. However, the response rate remains modest (around 50%)[13, 14]. According to their location and function, the many communication pathways that make up the microbiota-gut-brain axis can be manipulated by particular species of gut microbiota to

affect the brain and behaviour (which is the quickest way to manage homoeostasis). The gradients of oxygen, antimicrobial peptides, pH, and bile salts along the GI tract control the density and variety of microbial populations[15].

Past research have recorded many differences in sexes[1, 5, 6, 16] . Men are three times as likely than women to die by suicide. There are no known causes for the 43 percent increase in suicide rates among men aged 40 to 64 between 1999 and 2015[16]. One of suicide main coefficients is depression.

The gut microbiota's metabolites may either trigger enteroendocrine cells to release gut hormones or prevent them from doing so (such as serotonin) could penetrate the epithelial cell layer and enter the bloodstream, or stimulate the afferents that make up the vagus nerve, which communicate with the brain. Within the GI tract, numerous vagal afferents can be discovered that send multiple signals to the brain[15]. Thus, the nucleus of the solitary tract (NTS) is thought to regulate where and how information is sent out, resulting to various behavioural reactions, depending on the position of the microbiota throughout the gastrointestinal tract and the chemicals produced[15].

1.2 Statement of the Problem

Depression is a major public health concern in Nigeria, yet its genetic basis remains poorly understood. Despite available treatments, 30–60% of patients with Major Depressive Disorder (MDD) do not respond adequately, and remission rates are often below 50%. Antidepressant use is further complicated by adverse effects and harmful drug interactions[13]. Additionally, adolescent girls are nearly twice as likely to experience depression as boys, highlighting sex-based disparities[16]. The serotonin transporter gene (SLC6A4) has been implicated in treatment resistance, particularly in individuals carrying the short (S) allele. However, most studies have focused on non-African populations.

There is a critical need for genetic research within Nigerian cohorts to better understand MDD pathology and improve treatment outcomes through more personalized approaches[12, 13].

In the course of review of past research no studies dealt with the investigation of gonadocorticoids, serotonin levels and possible gut microbial presence or prevalence. Therefore, drawing correlations between genetic predisposing factors as well as hormonal imbalances to play roles in individuals suffering from depression of African decent is of importance.

1.3 Justification of the Study

The majority of the available data, points to a link between the short form of the 5-HTT and a higher likelihood of depressive syndrome after adversity[12]. Additionally, it's possible that an antidepressant won't have the same positive effects on these identical subjects. Young patients or those who are in the early phases of a depressive illness seem to be most sensitive to these effects. Therefore, early detection of these patients could influence antidepressant selection and result in better treatment outcomes[13]. A theoretical modelling study predicted that 64.6% of genotype-tested patients would be in remission at 6 weeks compared to 60.0% of individuals who were not tested for the gene[12, 13].

There is a need for additional treatment modalities with higher specificity to genetic backgrounds to be developed because the effectiveness of SSRIs in treating depression with a response rate that is still still moderate (about 50%)[13]. Understanding the genetic and biochemical pathogenesis may help in increasing the effectiveness and decreasing remission.

1.4 Aim and Objectives of the study

The aim of this study is to determine the effect the selected genes and biomarkers on people experiencing depression in Nigeria.

The specific objectives of the study are to;

i. Characterise the single nucleotide polymorphismS (SNPs0 (rs6354 and rs8076005) of SLC6A4 gene among people experiencing depression in Nigeria.

ii. Identify the copy number varitaion (CNV) (5HTTLPR) of SLC6A4 gene and associated blood serotonin levels among people experiencing depression in Nigeria.

iii. Determine blood serotonin levels in correlation to severity of MDD.

iv. Evaluate the blood pro-inflammatory cytokine (IL-6) levels across the severity of MDD patients.

v.Investigate the relationship between Gonadocorticoids (testosterone, progesterone and Estradiol) in MDD patients.

vi. Examine any synergistic association between these markers and severity of MDD.

1.5 Research Questions

Primary Research Questions include:

1. Is there a significant association between SLC6A4 gene polymorphisms (rs6354 and rs8076005) and depression severity in Nigerian patients?

2. What is the relationship between serotonin levels and depression severity in Nigerian patients?

3. Do gonadocorticoids play a role in modulating depression severity in Nigerian patients?

4. Is there a significant relationship between inflammation (IL-6 levels) and depression severity in Nigerian patients?

Secondary Research Questions that this research intended to resolve:

1. Are there sex differences in the relationship between SLC6A4 gene polymorphisms and depression severity?

2. Do lifestyle factors (alcohol consumption, smoking, employment status) influence the relationship between serotonin levels and depression severity?

3. Is there an interaction between gonadocorticoids and inflammation in modulating depression severity?

4. Can serotonin, gonadocorticoids, and IL-6 levels be used as biomarkers for depression diagnosis and severity in Nigerian patients?

1.6 Hypotheses

Null hypotheses: states that the SLC6A4 Gene (5HTTLPR, rs6354 & rs8076005) and Gonadocorticiods have no effects on the Serotonin Levels and Inflammation in Individuals Experiencing Depression.

Alternate hypotheses: states that the SLC6A4 Gene, (5HTTLPR, rs6354 & rs8076005) and, Gonadocorticiods have effects on the Serotonin Levels and Inflammation in Individuals Experiencing Depression.

1.7 Significance of the Study

Given current trends and socio-economic activities, it is important to demystify, elucidate and de-stigmatize depression. It has become increasingly important to observe the relationship between the SLC6A4 gene as a possible predisposing factor; serotonin levels; relationship between serotonin and its effects on inflammation, which will help in the better identification of genetic bio-markers as well as environmental variables in the treatment of this aggressive disease.Inflammation, in turn, is known to induce depression-like behaviour in animals and several symptoms of depression in many[14]. The therapeutic deficiency in treatment outcomes reflects the demand for revitalizing psychiatric

therapeutics with novel pharmaco-therapeutic options such as: pro-inflammation cytokine antagonists[12, 13].

1.8 Scope of the Study

This study investigated the relationship between the SLC6A4 long and short alleles, its polymporphs as they relate to serotonin levels, gonadocorticoids, inflammation, and demographic factors in the severity of depression in Nigerian patients.

The study population consisted of Nigerian patients diagnosed with depression, recruited from Psychiatric ward, National Hospital, Garki, Abuja; Neuropsychiatric Hospital, Rumigbo, Port Harchourt, Rivers State and Yaba Psychiatric. Samples were collected from Abuja, Lagos, and Port Harcourt, three major urban centers in Nigeria, due to their diverse and representative populations. These cities serve as key geopolitical and socio-economic hubs, attracting individuals from various ethnic, cultural, and linguistic backgrounds. As a result, they provide a heterogeneous population ideal for genetic studies, increasing the generalizability of findings across the broader Nigerian populace.

Additionally, these locations have well-established tertiary health institutions and psychiatric services, facilitating access to diagnosed cases of Major Depressive Disorder (MDD) and appropriate clinical support. The availability of infrastructure for ethical sample handling, storage, and transport further supported the selection of these sites for robust and reliable data collection.

This was a case-control study, where genetic information of the SLC6A4was used to observe various biomarkers that may influence occurrence of depression and its severity. Convenience sampling was used to determine sites for sampling, while participants were

recruited serially. The sample size consisted of 164 participants and was calculated using OpenEpi. Data was collected using questionnaires, blood samples were taken to measure neuroendocrine and inflammatory biomarkers. Data was analysed using EpiInfo. Hardy Weinberg equilibrium principle was used to ascertain the characteristtics of the study population. Graphs, charts and tables were used to present the data obtained in this study.

1.9 Limitations

While this study provided ground breaking genetic information for the Nigerian and the African population, the influence of other genetic and environmental factors were not fully explored.

This study had several limitations some of which included:

1. **Age Limitation**: The average age of participants significantly exceeds 25, possibly neglecting the experiences of younger individuals.
2. **Genetic Limitation**: The study examined only two SNPs (rs6354 and rs8076005), which may not encompass the complete spectrum of genetic variations linked to depression.
3. **Serotonin Measurement Limitation:** Serotonin levels were solely assessed in blood samples, which may not accurately reflect cerebral serotonin levels.
4. **Lack of Control Group Diversity**: The control group may lack sufficient diversity to accurately represent the general population.
5. **Absence of Longitudinal Data**: The study's design precludes analysing how variables change over time.

1.10 Operational Definition of Terms

Depression

Depression is a mental health disorder characterised by persistently depressed mood or loss of interest in activities, causing significant impairment in daily life.

Severity of Depression

Severity of depression is categorized based on HDRS scores:

Mild: 0-7

Moderate: 8-19

Severe: 20 or higher

Serotonin Levels

Serotonin levels are measured in nanograms per milliliter (ng/mL) using a standardized enzyme-linked immunosorbent assay (ELISA) kit.

SLC6A4 Gene

The SLC6A4 gene is defined as the gene encoding the serotonin transporter protein, with specific focus on the rs6354 and rs8076005 single nucleotide polymorphisms (SNPs).

Gonadocorticoids

Gonadocorticoids refer to the levels of testosterone and estrogen measured in nanograms per deciliter (ng/dL) using a standardized radioimmunoassay (RIA) kit.

Inflammation

Inflammation is measured by interleukin-6 (IL-6) levels in nanograms per deciliter (ng/dL) using the particular standardized ELISA kit for this research.

Demographic Factors

Demographic factors include:

1. Age: Measured in years

2. Sex: Categorized as male or female

3. Lifestyle factors: Measured using a standardized questionnaire assessing alcohol consumption, smoking habits, and employment status.

Endnotes

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Chapter Two

Literature Review

2.1 History of Depression

2.1.1 Earliest Account

Earliest records of what is now recognised as depression was written in Mesopotamia during the second millennium B.C.E. Depression was described in these literature as a spiritual rather than a physical disease. It was once thought that it was brought on by

demonic possession, just like other mental diseases. As a result, clergymen rather than doctors handled the situation[1].

2.1.2 Ancient Greek and Roman Philosophy

Many civilisations, including those of the ancient Greeks, Romans, Babylonians, Chinese, and Egyptians, held the belief that depression could have been brought on by demons and evil spirits. This notion caused it to frequently be treated using techniques including beatings, physical restraint, and fasting in an effort to drive the demons out. Greek and Roman physicians treated their patients using therapeutic techniques like massage, nutrition, music, baths, gymnastics, and a drug made of donkey's milk and poppy extract[2]. Greek physician Hippocrates hypothesised that four unbalanced bodily fluids known as humours yellow bile, black bile, phlegm, and blood were to blame for depression (originally referred to as "melancholia")[2]. He specifically believed that an excess of black bile in the spleen was causing melancholia. Hippocrates used blood-letting, baths, exercise, and di*et an*d preferred remedies[3].

On the other hand, a philosopher and statesman from Rome by the name of Cicero thought that melancholia had psychological origins like fury, anxiety, and grief [2].
Even educated Romans maintained the widespread idea that depression and other mental diseases were brought on by demons and the wrath of the gods in the final years before the common era[1, 2], despite some strides towards accepting more medical and mental causes of depression.

Many harsh and archaic methods of treating depression persisted during the common era. According to legend, Cornelius Celsus (25 BCE–50 CE) advised beatings, shackles, and starvation as extremely harsh cures for mental disease [4].

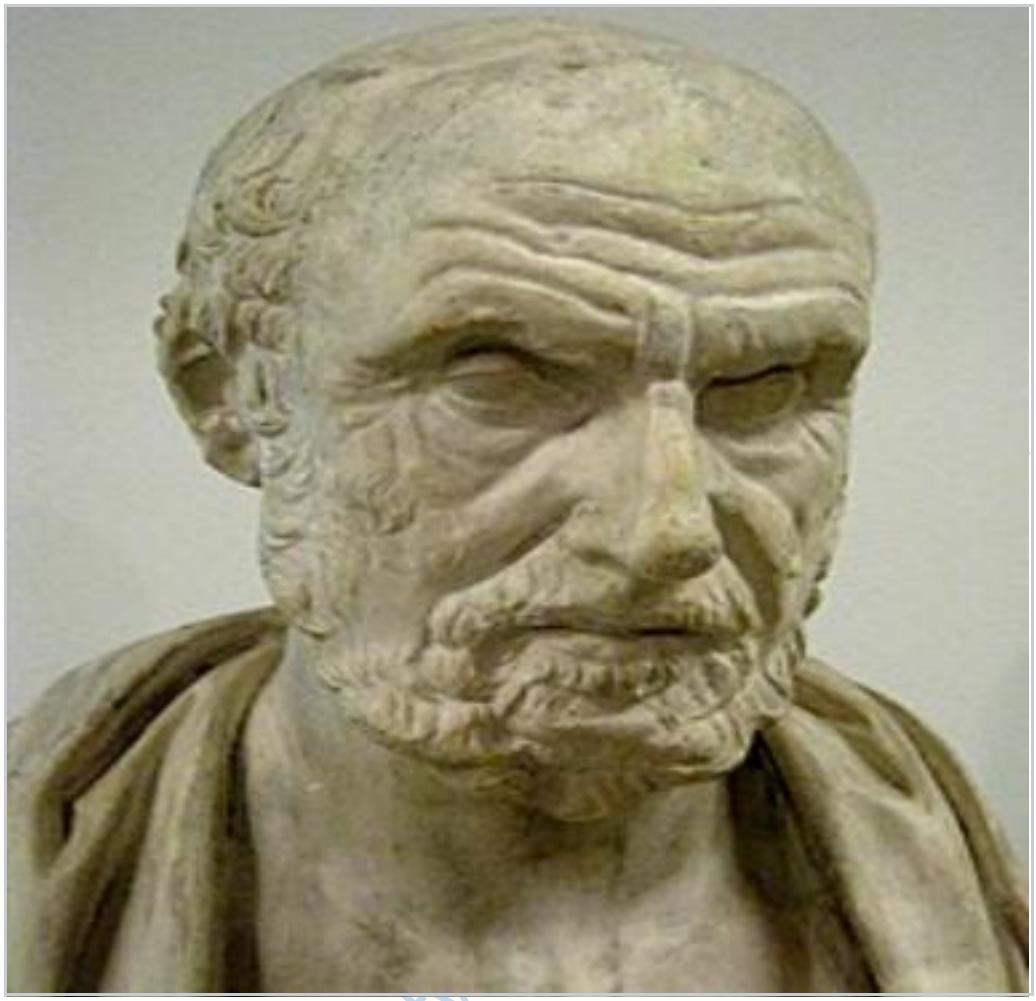


Plate 2.1: Pictorial Diagram of a Statue Greek Physician Hippocrates

Source[3]

2.1.3 Common Era

However, a Persian physician by the name of Rhazes (865–925 CE) did believe that mental illness is a brain disorder. He suggested therapies like baths and a very early type of behaviour therapy that used encouraging rewards for positive behaviour[5].

The Middle Ages saw a return to the practise of attributing mental disease to the devil, demons, or witches, with religion, especially Christianity, dominating European thought on the subject[5]. At the period, exorcisms, drowning, and burning were common cures. Many people were detained in places referred to as "lunatic asylums". these witch hunts were also common in the Renaissance era[6].

In the year 1621, Robert Burton published "Anatomy of Melancholy," in which he outlined the social and psychological causes of depression (such as poverty, fear, and loneliness)[7].

2.1.4 Age of Enlightenment

Depression started to be perceived as an innate temperamental weakness during the 18th and 19th centuries, commonly known as the Age of Enlightenment. These ideas led to the notion that those who had this illness should be isolated or imprisoned.

Doctors started to put out the theory that aggressiveness was the cause of the disease in the latter stages of the Age of Enlightenment[8].

During this time, therapies included submersion in water and spinning on a stool to realign the brain's components. Dietary adjustments, enemas (both of which are still recommended in modern medicine), horseback riding, and vomiting were other remedies. During this time, Benjamin Franklin is said to have created the first iteration of electroshock therapy[9].

Since Aristotle, melancholia has been seen as a risk of thought and creativity and has been connected to men of study and intellectual talent. Through the 19th century, the emerging notion abandoned these connotations and started to be increasingly connected with women[10].

2.1.5 19th and 20th Century

The first person to recognise manic depression, or what is today known as bipolar disorder, as a condition distinct from dementia praecox (at the time's term for schizophrenia), was the German psychiatrist Emil Kraepelin in 1895[11]. In the same period, the psycho-dynamic theory and the psychoanalysis-based psychotherapy method were developed.

In a 1917 essay on grief and melancholy, Sigmund Freud proposed the theory that melancholy is a reaction to loss, whether it be actual (such as a death) or symbolic (such as failing to attain the desired aim)[11, 12].

Freud also held the opinion that someone's unconscious resentment for a loss results in self-hatred and destructive behaviour. He believed that by assisting a person in resolving these unconscious disputes, psychoanalysis may lessen self-destructive ideas and actions[13].

The idea that behaviours are learnt via experience was influenced by the behaviourist movement in psychology. The behaviourists claimed that depression was a learned behaviour rather than the assumption that it was brought on by unconscious causes. A more effective, healthier set of behaviours could be established and strengthened using learning principles like association and reinforcement.

Cognitive theories of depression began to emerge in the 1960s and 1970s. According to cognitive theorist Aaron Beck, depressive symptoms may be influenced by how people understand traumatic situations[14].

Martin Seligman, a psychologist, proposed that depression might arise as a result of learned helplessness. This idea holds that people frequently give up on attempting to alter their circumstances because they believe that nothing they do will have an impact[10, 15]. The creation of cognitive behavioural therapy (CBT), which has been proven to be helpful in the treatment of depression, was significantly influenced by the rise of these cognitive models of depression.

The medical model of mental conditions first came into being in the 1970s and proposed that all mental disorders are primarily brought on by physiological reasons. Because mental health issues are treated with medicine in the same manner that physical ailments are, according to the medical model[10].

Depression can have biological causes that centre on things like genetics, brain chemistry, hormones, and brain structure[10] . This point of view was crucial in the creation and widespread application of antidepressants for the treatment of depression.

During the late 19th and early 20th centuries, treatments for severe depression weren't enough to help patients. Desperate for relief, many people turned to lobotomies, which are surgeries to destroy the brain's pre-frontal lobe to induce "calming" effect, lobotomies typically resulted in personality changes, a loss of judgement, bad decisions, and occasionally even death[16].

Patients with depression were occasionally treated with electro-convulsive treatment (ECT), which involves applying an electrical shock to the scalp to trigger a seizure. Doctors classified depression into sub-types of "endogenous," "neurotic," or "reactive" in the 1950s and 1960s[17]. While the neurotic or reactive kind of sadness was assumed to be the result of some external issues like a funeral or job loss, endogenous depression was thought to be caused by genetics or another bodily flaw.

Due to doctors' discovery that some patients' responses to the tuberculosis drug isoniazid appeared to be effective in treating depression, the 1950s were a significant decade in the treatment of depression[18]. Drug therapies are now beginning to be created and added to the mix of depression treatment, which had previously only included psychotherapy.

Tofranil (imipramine), one of the first medications to be developed for the treatment of depression, was followed by a number of other drugs referred to as tricyclic antidepressants (TCAs), which offered relief to many people but were frequently accompanied by serious side effects like weight gain, fatigue, and the possibility of overdosing[17, 19].

Following Prozac (fluoxetine), Zoloft (sertraline), and Paxil (paroxetine), other antidepressants entered the market. The brain's serotonin levels are the focus of these treatments, which are sometimes referred to as selective serotonin reuptake inhibitors (SSRIs) and typically have less side effects than earlier versions[17].

2.1.6 Modern Times

In recent times, the term major depressive disorder (MDD) was first introduced by clinicians in the United States during the 1970s, became part of the Diagnostic and

Statistical Manual of Mental Disorders (DSM-III) in 1980. Current edition of the diagnostic manual is the DSM-5 and is one of the primary tools used in the diagnosis of depressive disorders[17].

At the moment, medical professionals suggest that depression has a variety of underlying reasons, such as biological, psychological, and social ones, and that these causes are frequently cyclical. Additionally, clinicians are aware that some medical disorders can contribute to depression symptoms alongside other potential reasons like alcohol or drug abuse all of which must be ruled out[14].

In an effort to aid those who have not responded to therapy or medicine, novel therapies such as transcranial magnetic stimulation and vagus nerve stimulation have also been developed recently[17, 20].

Unfortunately, we do not fully comprehend the causes of depression, and no one treatment has been found to be effective for everyone. Mental health experts treat depression as a complex condition and have moved to recommend personalised treatment that includes medications, psychotherapies, and lifestyle modifications that are better suited to individuals.

2.2 Major Depressive Disorder (MDD)

The Diagnostic and Statistical Manual of Mental Disorders (DSM-5) fifth edition forms the foundation of the following definition of a major depressive episode:

Major depressive disorder (MDD), commonly referred to as clinical depression, A mental condition, recognised as is characterised by at least two weeks of consistently negative mood, low self-esteem, and loss of interest in or enjoyment from typically pleasurable activities. Since its adoption by the American Psychiatric Association for this symptom cluster under mood disorders in the 1980 edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-III), the phrase, which was first used by a group of US physicians in the middle of the 1970s, has been widely used[21].

Depression and depressive disorder are both frequent mental illnesses. It involves a persistently downcast attitude or loss of enjoyment or enthusiasm in activities.

Depression is distinct from typical mood swings and everyday feelings. It can have an impact on all facets of life, including interactions with friends, family, and the local community. It may be the cause of or a symptom of issues at work and in the classroom[2].

2.2.1 Other Types of Depression

The American Psychiatric Association's Diagnostic Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) has categorised depressive disorders into the following:

1. **Postnatal Depression:** is the term for the depression that can occasionally arise in new parents, fathers, or partners after they have a child[23].
2. **Persistent Depressive Disorder (Dysthymia)**: Mild to moderate depression that lasts for at least two years is referred to as PPD. Compared to major depressive illness, the symptoms are less severe. PDD was once referred to as dysthymia by medical professionals[23].

3. **Disruptive Mood Dysregulation Disorder (DMDD)**: In children, DMDD results in repeated episodes of extreme irritability and persistent irritability. Typically, symptoms appear around the age of 10[23].
4. **Depression brought on by a Different Medical Condition:** Numerous medical problems might alter your body in ways that lead to depression. Hypothyroidism, heart disease, Parkinson's disease, and cancer are a few examples. When the underlying illness is successfully treated, depression typically gets better as well[23].

Other special forms brought about by special conditions include:

1. **Seasonal Affective Disorder (SAD):** Commonly referred to as "winter depression," is a form of depression with a seasonal rhythm that is typically associated with winter or cold weather[24].
2. **Pre-Menstrual Dysphoric Disorder (PMDD):** A severe form of premenstrual syndrome (PMS), include depressive and anxious feelings in the weeks leading up to your menstruation[24].
3. **Atypical Depression:** This ailment, also referred to as major depressive disorder with atypical features, has symptoms that are a little different from those of "typical" depression. The primary distinction is a brief rise in mood in response to happy occasions (mood reactivity). Increased hunger and susceptibility to rejection are two additional significant symptoms[24].
4. **Bipolar Disorder**, also referred to as "manic depression," there are periods of both depression and an abnormally high mood (mania); the symptoms of depression are similar to those of depression, but mania episodes can include harmful behaviours like excessive spending, gambling, and unsafe sex[23, 24].
5. **Melancholic Depression (Melancholia)**: A severe subtype of MDD characterized by a profound loss of pleasure in almost all activities[23, 24].

6. **Psychotic Depression**: Major depression accompanied by psychotic features, such as delusions or hallucinations[24].
7. **Cyclothymic Disorder (Cyclothymia)**: A milder, chronic form of bipolar disorder. Symptoms: Fluctuations between hypomanic symptoms and depressive symptoms that don't meet full criteria for mania or major depression. Duration: At least 2 years in adults (1 year in adolescents/children). Often a precursor to Bipolar I or II[24].

2.2.2 Signs and Symptoms

The impact of major depression on a person's general health, work or school performance, family and personal connections, sleeping and eating patterns, and other activities is significant. A person experiencing a depressive episode typically displays the following symptoms: symptoms can include any of the following and may last for the majority of the day:

1. Sadness, emptiness, or a sense of futility.
2. Anxiety Feelings of unease, or worry.
3. Anhedonia- Loss of enjoyment or interest in the majority of everyday activities, including sex, hobbies, and sports.
4. Sleep disorders, such as insomnia or excessive sleeping, due to fatigue and a lack of energy, even simple tasks need more effort.
5. Feelings of guilt or worthlessness, a fixation on mistakes made in the past, or self-blame.
6. Irrational behaviour, irritation, or frustration, especially about trivial issues.
7. Weight loss and decreased appetite, or weight gain and increased desires for food.
8. Psycomotor retardation: sluggish speech, posture, or other body movements.

9. Problems with memory, concentration, decision-making, and thoughts of suicide, death, or other suicidal behaviour on a regular basis or repeatedly.
10. Constipation or irritable bowel syndrome.
11. Un-diagnosed bodily issues, such as back pain or headaches[25, 26].

All these signs must be observed everyday for at least two weeks.

For most people these symptoms are noticeable enough to affect the every day activities while some others only experience being miserable and unhappy with no explanations as to why.

2.2.2.1 Depression in Children And Adolescents

Although there may be some distinctions, the typical signs and symptoms of depression in adolescents and teenagers are comparable to those in adults.

1. Depression in young children might manifest as melancholy, impatience, clinginess, worry, aches and pains, refusal to attend school, or underweight.
2. Teens may experience symptoms such as sadness, irritability, feeling down and unworthy, anger, poor performance or poor attendance at school, feeling misunderstood and overly sensitive, using alcohol or drugs recreationally, eating excessively, engaging in self-harm, losing interest in regular activities, and avoiding social interaction[27, 28].

2.2.2.2 Depression in Senior Citizens

Depression is never to be taken lightly or as a typical aspect of ageing. Unfortunately, older persons with depression frequently go undetected and untreated, and they may be hesitant to get care. Older persons may experience various or less noticeable signs of depression, such as:

1. Memory issues or character alterations.
2. Physical discomfort.
3. Symptoms of exhaustion, anorexia, insomnia, or loss of desire in sex that are not brought on by a disease or medication.
4. Frequently preferring to stay in rather than leave the house to interact with others or try new things.
5. Especially with elderly men, suicidal thoughts or feelings[29].

Severities of Depression are based on he symptoms, including how often you get symptoms and how bad they are, how long depression lasts and the impact on your daily life.

2.2.3 Causative Agents

Considering that the true aetiology of depression is not fully understood:

1. Neurotransmitter levels in the brain: Serotonin and dopamine are two neurotransmitters that are thought to be imbalanced in depression.
2. Genetics: Individuals that are three times as likely to experience depression as the general population are those that have a first-degree relative (a biological parent or sibling) who has the illness. But depression can exist even when there is no familial history of the condition.

3. Stressful Life events : Difficult situations including the death of a loved one, trauma, divorce, loneliness, and a lack of support can cause depression.
4. Chronic pain and long-term illnesses like diabetes can contribute to sadness.
5. Medication: Depression is a side effect of some drugs. Alcohol use is one substance that can either induce depression or exacerbate it.
6. Early substance use has been linked to a higher risk of depression later in life.
7. Childhood trauma, including neglect, abuse, and dysfunction in the family[22, 30, 31].

2.2.4 Risk Factors

Some people pose higher risks of depression than others, due to certain conditions they are exposed to. Risk factors include:

1. Experiencing specific life events, such as a death in the family, challenges at work, relationship changes, money troubles, and health issues
2. Lacking effective coping mechanisms
3. Having a close family member that is depressed.
4. Experiencing acute stress.
5. Prescription medications such as corticosteroids, certain beta-blockers, and interferon
6. Smoking, using alcohol or amphetamines as recreational substances
7. Accidents that cause brain injury.
8. Having a neurological condition like Parkinson's or Alzheimer's.
9. Having experienced a recent depressive episode in the past
10. Having a chronic illness or disorder, such as hyper/hypothyroidism, chronic obstructive pulmonary disease (COPD), or cardiovascular disease.
11. Having persistent pain (back pain).
12. Limited social support[21, 22].

Pathophysiology

Current theories on the pathophysiology of depression concentrate on monoaminergic systems, circadian rhythm, immunological dysfunction, HPA-axis dysfunction, and structural or functional abnormalities of emotional circuits. However, there is still much to learn about these topics.

The monoamine theory asserts that low levels of monoamine neurotransmitter activity are the root cause of depression and was inspired by the success of monoaminergic medications in treating depression[32] . Multiple sources provide support for the monoamine idea.

First, tryptophan deficiency, a crucial precursor to serotonin and a monoamine can result in depression in those who are not depressed or in close family members of those who are, indicating that reduced serotonergic neurotransmission is significant in depression.Second, there appears to be a connection between polymorphisms in the 5-HTTLPR gene, which codes for serotonin receptors, and the chance of developing depression. Third, data from rat models and decreased adrenergic neurotransmission in depression are suggested by smaller locus coeruleus, decreased activity of tyrosine hydroxylase, increased density of alpha-2 adrenergic receptor, and decreased tyrosine hydroxylase activity[18, 32, 33, 34].

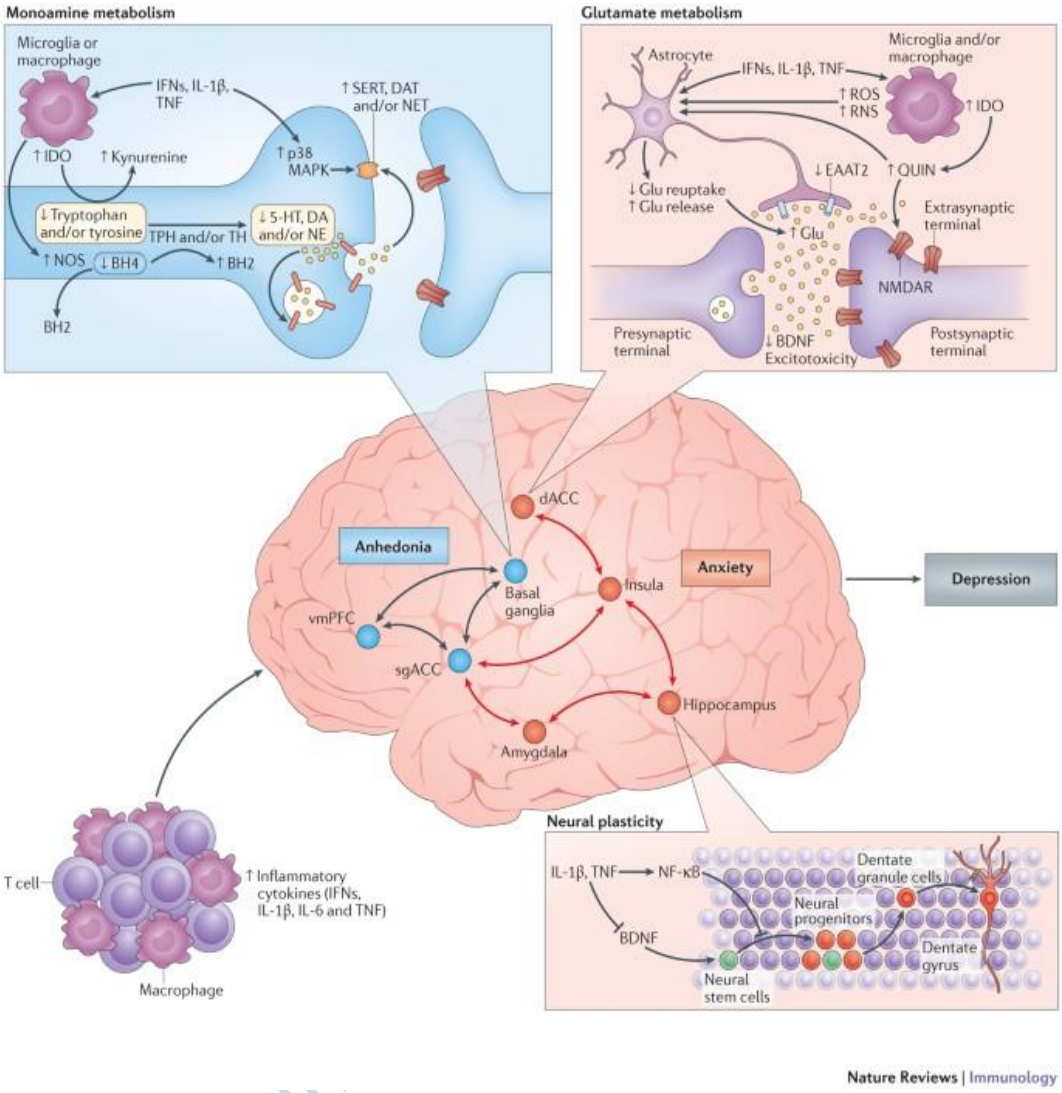


Figure 2.2: Pathophisiology of Depression

Source[33]

Dopamine, a different monoamine, is also implicated in depression due to decreased levels of homovanillic acid, altered responses to dextroamphetamine, responses of depressive symptoms to dopamine receptor antagonists, decreased dopamine receptor D1 binding in the striatum, and polymorphism of dopamine receptor genes[35].

Finally, depression has been linked to increased monoamine oxidase activity, which breaks down monoamines.The observation that serotonin depletion does not result in depression in healthy individuals, the fact that antidepressants immediately increase monoamine levels but take weeks to start working, and the existence of atypical antidepressants that can be effective despite not targeting this pathway all cast doubt on the monoamine theory[34, 35].

One theory for the therapeutic lag and more evidence for the monoamine shortage is that antidepressants' higher serotonin levels desensitise self-inhibition in raphe nuclei[18, 27, 33]. A depressed state mediated by elevated serotonin has been postulated to result from disinhibition of the dorsal raphe, which is thought to happen as a result of reduced serotonergic activity in tryptophan deficiency.

The fact that rats with dorsal raphe lesions are not more depressed than controls, the discovery of increased jugular 5-HIAA in depressed individuals that normalised with selective serotonin re-uptake inhibitor (SSRI) treatment, and the preference for carbohydrates in depressed individuals all work against the monoamine hypothesis. The monoamine hypothesis is already limiting, and when it is explained to the general public, it is further oversimplified[32, 33]. No consistent evidence was discovered in 2022 to back up the serotonin hypothesis, which links low serotonin levels to depression[36].

Increased amounts of cytokines that cause illness behaviour (which overlaps with depression) have been linked to immune system disorders. Interleukin-6 (IL-6), TNF-, IL-10, and the C-C motif ligand 2 chemokine were all significantly lower in the peripheral circulation after taking antidepressants, indicating that these markers of peripheral inflammation may also be decreased[37, 38].

The efficacy of non-steroidal anti-inflammation drugs (NSAIDs) and cytokine inhibitors in the treatment of depression[18], as well as the normalisation of cytokine levels following a successful course of therapy, further point to immune system abnormalities in depression[38].

Given the relationship of CRHR1 with depression and the increased incidence of dexamethasone test non-suppression in depressed individuals, HPA-axis anomalies have been proposed in depression. Since this abnormality's sensitivity is approximately 44%, it is insufficient as a diagnostic tool. Hippocampal volume reductions observed in depressed individuals are assumed to be the result of these stress-related anomalies[38].

Dexamethasone suppression was also lessened, and the reaction to psychological stresses was enhanced, according to a meta-analysis. The cortisol awakening response, whose elevated reaction is linked to depression, has hidden additional aberrant results[39].

The results of neuroimaging have been unified by theories. The ventral para-limbic regions are hyperactive and the frontal regulatory regions are hypoactive in the limbic-cortical model, which is the first model put forth. A different theory, known as the cortico-striatal model, contends that depression is caused by anomalies in the pre-frontal

cortex's control of the striatal and sub-cortical systems. Another paradigm, which is in line with studies on emotional bias, suggests that salience structures are hyperactive in recognising negative stimuli while cortical regulation structures are hypoactive, leading to a negative emotional bias and depression[40].

2.2.5 Diagnosis

A psychiatrist or psychologist with the necessary training may do a diagnostic examination after taking the patient's current situation, biographical history, present symptoms, genealogy, and substance abuse into account. The evaluation also involves a mental state examination, which evaluates the subject's present attitude and thought patterns, particularly the presence of themes of despondency or pessimism, attempted suicide or self-harm, and the absence of optimistic ideas or goals[25]. Since there are few specialist mental health services available in rural locations, primary-care practitioners are typically the ones responsible for diagnosis and treatment. The severity of this problem is greater in developing nations.

Although rating scales are not used to diagnose depression, they do give an indicator of the severity of symptoms over time, allowing those who score above a certain cut-off point to have a more complete evaluation before receiving a diagnosis of depressive illness. The Hamilton Rating Scale for Depression, the Beck Depression Inventory, the Suicide Behaviours Questionnaire-Revised, and other rating measures are used for this purpose[21].

Compared to psychiatrists, primary-care physicians struggle more with the inadequate awareness and under-treatment of depression. Since some patients with depression frequently experience physical symptoms in addition to their despair, these situations could go unnoticed. Additionally, there might be obstacles relating to the patient, the

provider, or the medical system. According to studies, non-psychiatric doctors overlook roughly two-thirds of instances[22].

In order to rule out other potential causes of depressive symptoms, a doctor typically conducts a medical examination. These include blood tests for fundamental electrolytes and serum calcium to rule out a metabolic disruption, blood tests for TSH and thyroxine to rule out hypothyroidism, and a complete blood count with ESR to rule out a systemic infection or chronic disease. Additionally, it may be possible to rule out alcohol abuse or adverse drug effects[21]. Men who suffer from depression due to hypogonadism may have their testosterone levels checked. Since low vitamin D levels have been linked to an increased risk for depression, vitamin D levels could be measured[41].

Older depressed persons often experience subjective cognitive symptoms, but they can also signal the beginning of a neurological pathology like Alzheimer's disease. Differentiating between depression and dementia can be aided by cognitive tests and brain imaging. In people with psychotic, sudden-onset, or other odd symptoms, a CT scan can rule out brain pathology[21, 23].

The Diagnostic and Statistical Manual of Mental Disorders (DSM) and the International Statistical Classification of Diseases and Related Health Problems (ICD) published by the World Health Organisation and American Psychiatric Association, respectively, contain the diagnostic criteria that are most frequently used to identify depressive disorders[42].

Other possible diagnoses, such as dysthymia, adjustment disorder with low mood, or bipolar disorder, must be taken into account in order to rule out major depressive disorder as the most likely diagnosis[23].

2.2.6 Prevention

Although depression cannot be prevented, it can be reduced by the following:

1. Maintaining a healthy sleep routine.
2. Managing stress with healthy coping mechanisms.
3. Practising regular self-care activities such as exercise, meditation and yoga.
4. Maintaining a good di*et an*d avoiding alcohol which is an antidepressant.
5. Going to "talking" therapy especially when individuals are experiencing adversity in life either to professionals or family members among others.

2.2.7 Treatment

Those with the most severe symptoms or forms of depression are treatable. Treatment is more successful the earlier it is started. Typically, psychotherapy, drugs, or a combination of the two are used to treat depression[25, 27, 28, 33, 34, 36, 37, 38, 39].

When a person tries at least two antidepressant drugs without improving, they may have treatment-resistant depression. Brain stimulation therapy may be a possibility to investigate if conventional therapies like psychotherapy and medication fail to lessen depressed symptoms or if there is an urgent need for quick symptom relief.

The following are drugs for MDD that have FDA approval: While the effectiveness of each antidepressant is the same, their side-effect profiles vary:

1. **Selective Serotonin Re-uptake Inhibitors (SSRIs)**: Citalopram, fluvoxamine, sertraline, escitalopram, paroxetine, and fluoxetine are a few examples. They are the most often prescribed antidepressants and are used as the first line of treatment [33].

2. **Serotonin-Norepinephrine Reuptake Inhibitors (SNRIs)** include: Milnacipran venlafaxine, levomilnacipran, duloxetine, and desvenlafaxine. They are frequently administered to people with depression and concurrent pain conditions [34].
3. **Serotonin Modulators** such as: Vilazodone, trazodone, and vortioxetine are Bupropion and mirtazapine are two examples of atypical antidepressants. When patients experience sexual side effects from taking SSRIs or SNRIs, doctors frequently prescribe them as "mono-therapy" or as enhancing medications [33,34].
4. Clomipramine, amitriptyline, imipramine, nortriptyline, doxepin and desipramine are **Tricyclic Antidepressants (TCAs)**[25, 27, 28, 33, 34, 36, 37, 38, 39].
5. **Monoamine Oxidase Inhibitors (MAOIs)** examples are: Tranylcypromine, selegiline, phenelzine, and isocarboxazid, that are on the market however, due to the high frequency of side effects and mortality in overdose, MAOIs and TCAs are not frequently utilised[32].
6. Other drugs, such as mood stabilisers and anti-psychotics, may be given to improve the effects of antidepressants[43].

Antidepressants take time usually 4-8 weeks to work, and problems with sleep, appetite, and concentration often improve before mood lifts.

1. **Psychotherapy** such as**:** psychological counselling, interpersonal counselling[43].
2. **Electroshock Therapy (ECT)** is used in cases were individuals suffering from serious symptoms which include: Serious suicidality, obstetric depression of great severity, refusal of food or drink, Catatonia, extremely psychotic. In order to cause a seizure, which alters the electrical activity in the brain, this treatment delivers electrical currents to the brain. During the process, you won't feel a thing because you'll be completely asleep. Remember that ECT now differs greatly from "shock therapy" used in the middle of the 20th century.

3. **Transcranial Magnetic Stimulation (TMS):** FDA-approved for individuals who have tried at least one medication but still have treatment-resistant or refractory depression. This method sends magnetic impulses into your head in an effort to activate brain function and nerves[43, 44, 45, 46, 47, 48, 49].
4. **Vagus Nerve Stimulation(VNS):** It is thought that this treatment, which uses a device placed in your chest to stimulate your vagus nerve, can help balance the brain chemicals associated with depression. FDA-approved as a long-term supplementary depression treatment for people who have tried at least four different medications without success [45, 46,].
5. **Esketamine:** For patients who have not responded to other antidepressants, esketamine nasal spray should be administered in addition to an oral antidepressant in cases of treatment-resistant depression [44, 45, 46].
6. **Other Supplements:** Studies are still underway, some people treat depression with natural remedies like vitamin D, saffron, magnesium and the herbal dietary supplement St. John's wort.[41]
7. **Light Therapy:** People with seasonal affective disorder frequently opt for daily morning light therapy as a form of treatment[43, 44, 45, 46, 47, 48, 49].

Medications used to treat depression frequently have side effects. The particular alterations you might go through are partially influenced by the drug class prescribed.

2.2.7.1 Typical Side Effects

Side effects that cut across must of the medications include:

1. Indigestion, diarrhoea, constipation, and loss of appetite are among the gastrointestinal symptoms;
2. Alterations in heart rhythm: palpitations, rapid pulse;
3. Agitation, trembling, and an anxious feeling of nervousness;
4. Vision alterations: hazy vision;
5. Low sex desire is a sexual disorder;
6. Changes in sleep: insomnia;
7. Unexpected weight changes, such as weight gain or loss;
8. Other symptoms include a headache, vertigo, dry mouth, and sweating

Any, all, or none of could be potential side effects[50].

2.2.7.2 Potential Hazards to Health

More serious side effects include:

1. **Serotonin Syndrome**: develops when serotonin levels are too high, typically as a result of using antidepressants with serotonin re-uptake inhibitors. Confusion, jerking muscle seizures, an irregular heartbeat, or unconsciousness are symptoms[51].
2. **Hyponatremia**: In older persons using antidepressants, hyponatremia (low blood sodium) is a hazardous decline in salt levels in the body. Headache, muscle soreness, confusion, agitation, or convulsions are some of the symptoms[51].
3. **Diabetes**: Type 2 diabetes may be more likely to develop in antidepressant users[51].
4. **Suicidal Ideation**: When taking antidepressants for the first time, some people, especially those who are younger, may consider harming themselves. You can *get as*sistance from your doctor, a hotline, or the closest emergency room[51].

5. Compared to typical antidepressant side effects, these health hazards don't occur as frequently. They are all significant ailments for which you can seek assistance from a physician or other mental health expert[51].

2.2.8 Prognosis

Major depressive disorder's untreated depression episodes can endure for up to a year. About 15% of those with moderate to severe depression actually commit suicide, and about two-thirds of those people think about it. Being a chronic, recurrent condition, MDD has a 50% recurrence rate after the first episode, a 70% recurrence rate after the second episode, and a 90% recurrence rate after the third incident[49, 52] . Bipolar illness develops in almost 10% of MDD individuals over time. Patients with moderate episodes, no psychotic symptoms, improved treatment compliance, a robust support system, and adequate pre-morbid functioning have a positive prognosis for MDD. When a personality disorder, multiple hospitalisations, co-morbid mental disorders, and advanced age of ons*et ar*e present, the prognosis is poor[49, 52].

2.2.9 Statistics

2.2.9.1 Statistics of Depression Around the World

According to WHO's predictions, major depressive disorder (MDD) would overtake all other diseases by 2030 and be the third leading source of illness globally. Approximately 3.8% of people in the population suffer from depression, including 5.7% of people over 60 and 5% of adults (4% of males and 6% of women)[52].

Around 280 million people worldwide suffer from depression. Women are around 50% more likely than men to experience depression. More over 10% of pregnant and recently delivered women experience depression globally. Every year, around 700,000 people

commit suicide. For people aged 15 to 29, suicide is the fourth most common cause of death[53].

Over 75 % of people in nations with low or middle incomes do not obtain therapy, despite the fact that there are well-established, efficient treatments for mental diseases[22, 53].

In the United States, approximately 21.0 million adults experienced a minimum of one major depressive episode. This figure corresponded to 8.3% of all American adults. Adult females had a higher rate of major depressive episode (10.3%) than adult males (6.2%). Adults aged 18 to 25 made up the largest proportion of those who had experienced a major depressive episode (18.6%)[22, 25]. Those who reported having many (two or more) races had the highest rate of major depressive episode (13.9%).

According to estimates, 61.0% of American individuals 18 and older who had a major depressive episode in the previous year received treatment in 2021. An estimated 74.8% of people with major depressive episode and significant disability received treatment in the previous year. An estimated 44.2% of teenagers with a major depressive episode and significant impairment received treatment in the previous year[22, 25].

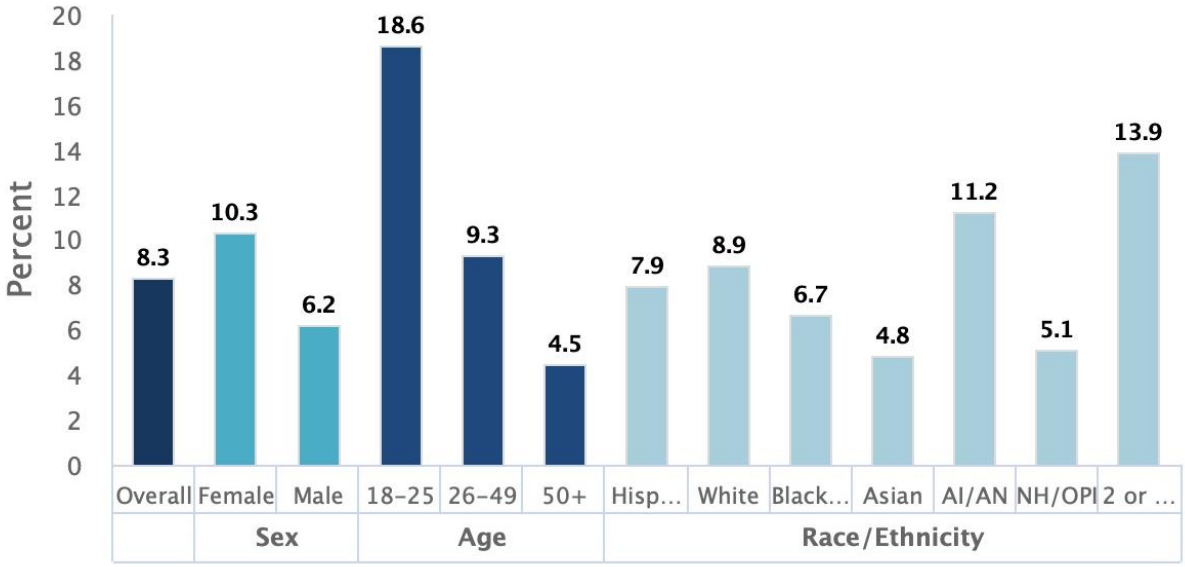


Figure 2.3: Statistics of Major Depressive Disorder among American Adult Population (2021)

Source[42]

2.2.9.2 Statistics of Depression in Nigeria

With a prevalence rate of 3.9%, depression is a serious public health issue that affects 7 million Nigerians today[55]. About 20% to 59% of people living with HIV/AIDS are depressed, with North-Central Nigeria reporting the highest incidence. Depression is

prevalent in young adults, the elderly, and IDPs, with prevalence rates of 25%, 26.2%, and 17%, respectively. Up to 44.5% of clinical patients, according to a 2013 study carried out in Western Nigeria, are depressed. WHO estimates that women experience 50% more depression than men do. In Africa, 5.95% of females and 4.9% of males experience depression[55].

Studies conducted in Nigeria have revealed that being of the female gender increases the likelihood of developing depression. In Nigeria, 28% and 7% of females, respectively, reported experiencing both physical assault in their lifetimes. Additionally, over 50% of women are illiterate and out of the labour force. According to studies, 14% to 20% of new mothers in Nigeria exhibit this condition[55].

Studies show that this disease is present in 14% to 20% of first-time mothers in Nigeria. underlines how crucial society and culture are to the widespread prevalence of health problems in the nation[54].

According to a study, the majority of medical staff at a Benin City medical facility have little awareness of depression and struggle to treat patients who are depressed in 78% of cases. This suggests that general physicians and other healthcare professionals need to be appropriately and consistently made aware of depression and additional psychological illnesses. Additionally, there is a lack of information about depression along with other mental health issues, which highlights the need for more study in this field, particularly in the community where information is required for effective health planning[55].

2.3 The Brain- The Vagus Nerve- The Gut

In times of excellent health, the host and the 100 trillion microorganisms that make up the gut microbiota form a symbiotic connection. 90% of the microbiota is made up of the two bacterial phyla Firmicutes and Bacteroidetes. The growth of the gut microbiota can be influenced by factors such as genes, age, sex, nutrition, life events, the environment, and stress levels. Nervous system, including response to stress, mood and anxiety states[56]. The microbiome affects immunity, vitamin synthesis, intestinal barrier permeability, and digestion. Through the gut-brain axis, the gut microbiota also affects the brain in the opposite direction. The gut-brain axis, which is implicated in hormonal, immunological, and neurological homoeostasis, refers to the bidirectional communication between the gut and the brain. This implies that alterations in gut bacteria can have an impact on the central nervous system (CNS), including response to stress, mood and anxiety states[56, 57].

A significant portion of the brain-gut-microbiota axis is made up of the network of neurological signal, immunological signals and chemical signals. Changes in this network result in intricate interactions between organs via direct (nerves) and indirect (systemic circulation) routes, which affects the body's homoeostasis. Depression can be brought on by changes in the gut microbiota's composition and in the S CFAs, D-amino acids, and metabolites produced by the microbiome[58].

Gamma-aminobutyric acid (GABA) is produced by some strains of Bifidobacterium and Lactobacillus, while serotonin is known to be produced by Escherichia and Enterococcus. As shown in F**igure 2.4**, the generation of bacterial metabolites such short-chain fatty acids (SCFAs) by the gut microbiota can also stimulate the CNS[56].

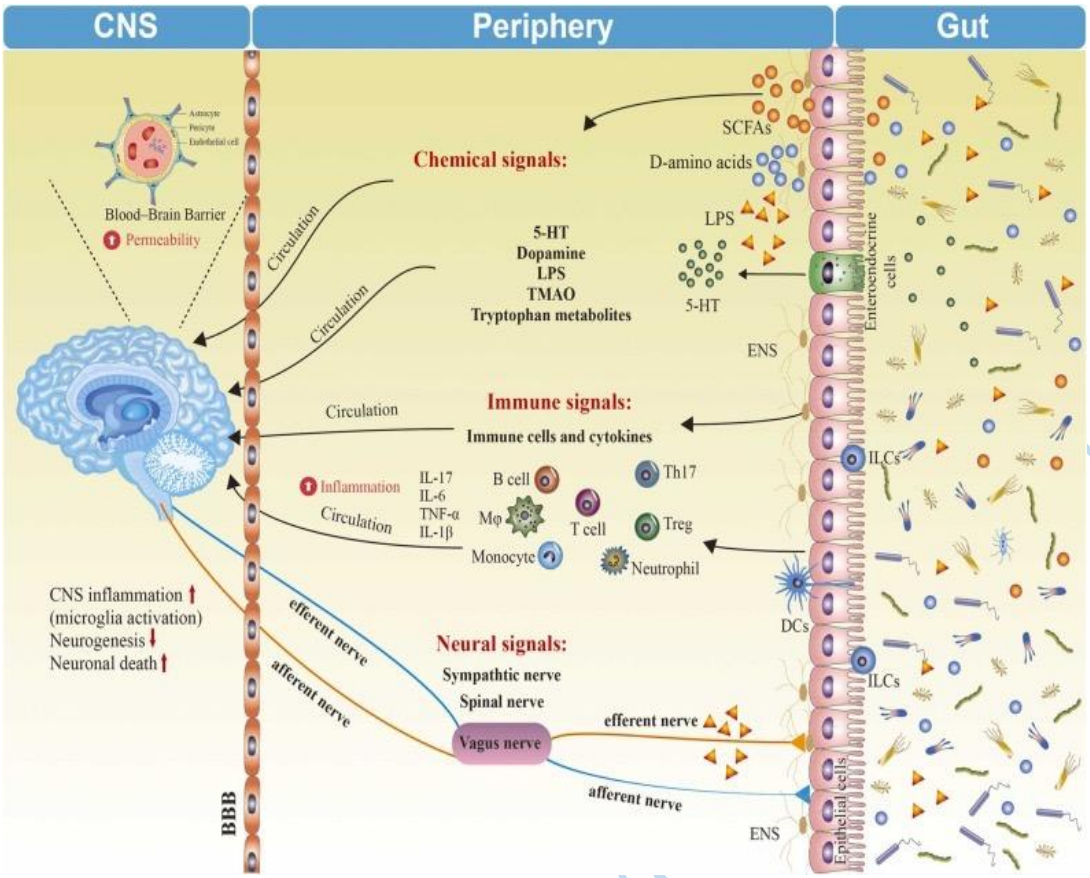


Figure 2.4: [The Brain–Gut–Microbiota in Depression]

Source[56]

the function of the body's brain-gut-microbiota axis. The central nervous system (CNS), the peripheral, and the gut are illustrated as the three main axes of the brain-gut-microbiota axis. A significant portion of the brain-gut-microbiota axis is made up of the network of neurological signals, immunological signals, and chemical signals. Changes in

this network result in intricate interactions between organs via direct (nerves) and indirect (systemic circulation) routes, which affects the body's homoeostasis[56].

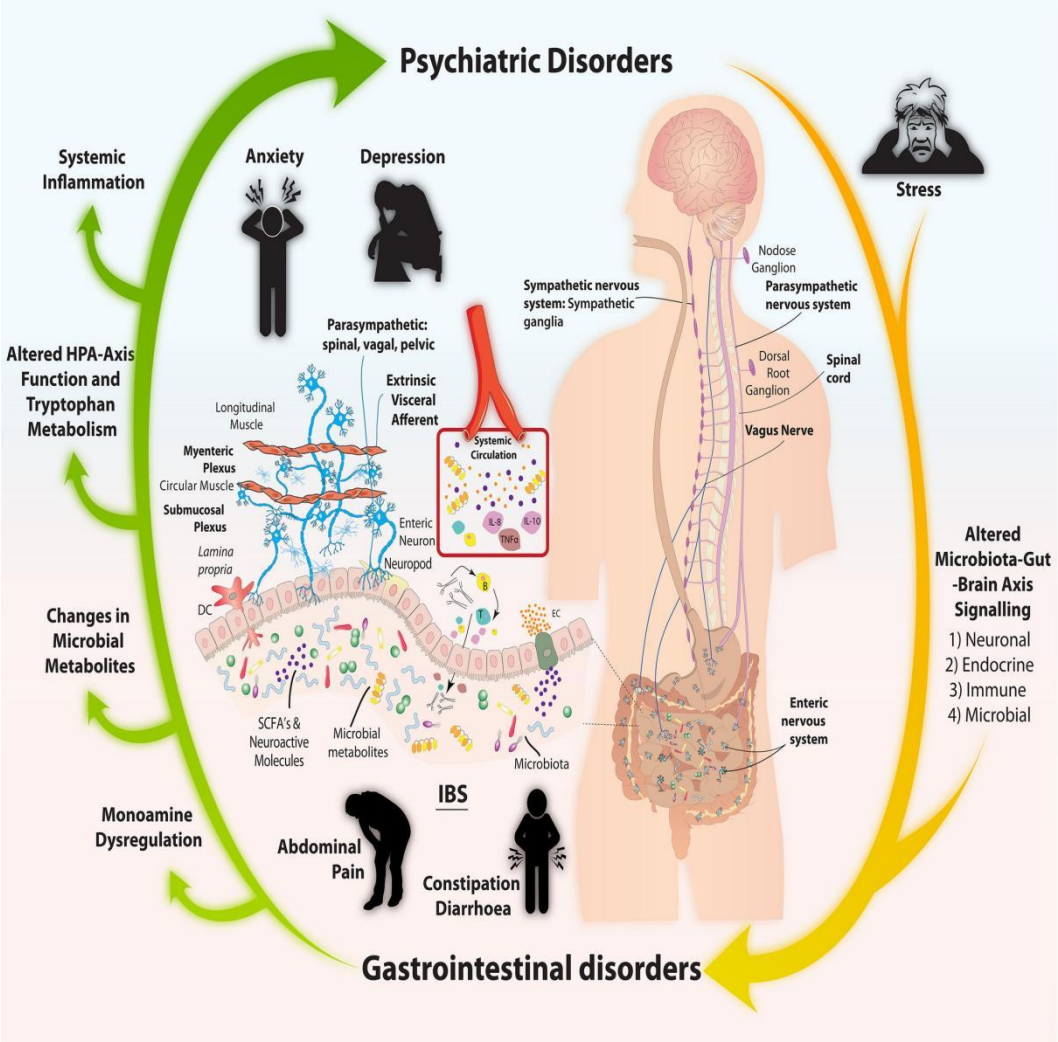


Figure 2.5: Central Nervous System (CNS) and Gut Cycle in Depression

Source[58]

The central nervous system (CNS), the peripheral, and the gut are illustrated in F**igure 2.4 and 2.5** as the three main axes of the brain-gut-microbiota axis. A significant portion of the brain-gut-microbiota axis is made up of the network of neurological signals,

immunological signals, and chemical signals. Changes in this network result in intricate interactions between organs via direct (nerves) and indirect (systemic circulation) routes, which affects the body's homoeostasis[57].

Depression can be brought on by changes in the gut microbiota's composition and in the SCFAs, D-amino acids, and metabolites produced by the microbiome. The CNS controls peripheral organs and tissue through neuronal regulation, neurotransmitters, immunological signals, and other elements in response to various signal reflexes, all of which contribute to appropriate bidirectional brain-gut axis signalling[58].

Coprococcus and Faecalibacterium. Endocrine cells, the immunological system, and neurons are the targets of SCFAs[60]. Through a variety of channels, including immunological signals, hormones, and the vagus nerve, the SCFAs probably mediate interactions between the gut and the brain [56]. Additionally, SCFAs directly activate tryptophan hydroxylase, causing intestinal enterochromaffin cells to produce serotonin (5-HT).

In order to control motility, serotonin is released through neurons in the enteric nervous system, and it is thought to be a key mediator of the gut-brain axis. It is still unclear exactly how gut serotonin interacts with the brain. According to one theory, serotonin (5-HT3, 5-HT4) and other metabolites produced by bacteria are receptors on the vagus nerve fibres. The bulk of 5-HT neurons are found in the nucleus of the solitary tract, which is where these receptors enable the vagus nerve to detect microbial signals and communicate with the brain. It's interesting to note that SCFAs can cross the blood-brain barrier (BBB), although GABA and serotonin don't seem to be able to do so until there is inflammation, which may change the BBB's permeability[61].

'Depressed and stressed' rodents had notably abnormally low amounts of SCFAs in their intestines[56]. These results are in line with the Flemish Gut Flora Project, a significant human microbiome study (n=1054) that revealed decreased numbers of butyrate-producing species Coprococcus spp. and Dialister in individuals with MDD[59]. In this study, higher quality of life measures were consistently correlated with higher concentrations of butyrate-producing Faecalibacterium and Coprococcus bacteria[60].

Additionally, higher levels of IL-1, which has been linked to severe neuroinflammation and BBB leak in animal models, were found in MS patients with severe depressive episodes[62]. Additionally, because TNF can stimulate the hypothalamus-pituitary-adrenocortical (HPA) and Indoleamine 2,3-dioxygenase (IDO), causing a decrease in tryptophan production has been associated to underdevelopment abnormalities, inflammation-related neurodegenerative illnesses, and depression[62].

Pro-inflammatory cytokine receptor expression has been found to be elevated in the peripheral blood and cerebrospinal fluid (CSF) of MDD patients, a finding that can be reversed with antidepressant therapy[63]. Additionally, post-mortem examination brain samples from suicide subjects who had depression were shown to include the genes for innate immune proteins like IL-1, IL-6, TNF, Toll-like receptor 3 (TLR3), and Toll-like receptor 4 (TLR4)[62]. Those facts propose that cytokines are crucial biomarkers of MDD.

Additionally, immune cells are crucial to the pathophysiology of MDD. Macrophages are cells in the immune system that regulate inflammation to maintain homoeostasis. They can be classified as traditionally activated cells (M1) or alternatively activated cells (M2), which secrete cytokines that promote inflammation or tissue repair, respectively. There is a change in macrophage populations towards the M1 phenotype in any illness state of the

CNS disorders with inflammation, which are significant contributors to inflammation neurodegeneration in individuals with severe depression[64]. A pro-inflammatory "M1" macrophage phenotype and an over-expression of IL-6 are evident in the peripheral blood gene expression profiles of people with depression[65].

2.4 Serotonin: The Neurotransmitter

Serotonin a monoamine neurotransmitter is involved in a number of intricate biological processes[66]. Given that 5-hydroxytryptamine is its chemical name, it is frequently abbreviated as 5-HT. It is believed that Vittorio Erspmarer accidentally discovered serotonin in 1935 while attempting to refine an extract from enterochromaffin cells[67].

The amino acid tryptophan is hydroxylated (i.e., given an extra -OH group) and decarboxylated to produce serotonin. The enterochromaffin cells of the digestive tract contain the most serotonin, with minor levels being present in the CNS system and platelets. By acting on serotonergic receptors, which are connected to several G proteins that mediate intracellular changes, serotonin causes changes in the cell.

Researchers were searching for a chemical that platelets emitted that caused vasoconstriction when they discovered serotonin. Following its discovery, it was also initially known as enteramine because, after being released from enterochromaffin cells, it causes smooth muscle contraction in the gastrointestinal system[68].

Later, it was discovered that the human brain also releases serotonin as a neurotransmitter. The serotonergic system was named after these excretory neural clusters. Serotonin has a wide range of roles in the central nervous system (CNS), many of which are related to how the serotonergic system affects the forebrain, brainstem, and cerebellum[66]. This

system's rostral nuclei project signals that control body temperature, appetite, sleep patterns, emesis, and sexual behaviour.

Serotonin's role in mental diseases is most clinically significant; typically, depression, anx iety, and mania are associated with its lack[69, 68]. Following its discovery, it was also initially known as enteramine because, after being released from enterochromaffin cells, it causes smooth muscle contraction in the gastrointestinal system[68].

The body contains seven different kinds of serotonin receptors[66]. The majority of subtypes are heterogeneous and can be further broken down into 5-HT1A, 5-HT2B, 5-HT3, etc. In six of these subtypes, G-protein-coupled receptors are involved.

Gamma-aminobutyric acid (GABA) and N-methyl-d-aspartic acid (NMDA) both include ligand-gated Na/K ion channels, but the 5-HT receptor is distinct in that it does as well[69]. Adenylyl cyclase and the 5-HT1 and 5-HT5 receptors interact adversely, and the activation of these receptors inhibits the production of cyclic AMP. The release of intracellular Ca is caused by the 5-HT receptor's upregulation of the inositol triphosphate and diacylglycerol pathways. Adenylyl cyclase is triggered by the interaction of 5-HT4, 5-HT6, and 5-HT7 receptors, which raises cAMP levels[68].

When 5-HT and the Na/K cation channel interact, the plasma membrane depolarizes. The recapture of 5-HT from the cells synapses aids in the cessation of serotonergic activity. Within the presynaptic neurons (serotonergic, pineal, and catecholaminergic neurons) of the central nervous system (CNS), serotonin is produced and stored. Nine distinct groupings of cell bodies in the pons and midbrain are serotonin-rich. The primary nuclei are the raphe nuclei, which also contain descending fibres that reach to the medulla and spinal cord in addition to ascending serotonergic fibres that project to the forebrain. There

are also a few serotonergic nuclei in reticular development with fibres still present in the medulla[70].

Serotonin is processed in the CNS in a number of different ways. Serotonin enters the synaptic cleft as a result of neuronal depolarization. It can attach to presynaptic serotonin autoreceptors or postsynaptic serotonin receptors (5-HT receptors)[72].

Serotonin binding to the autoreceptor functions as a brake on future serotonin release into the synaptic cleft. Serotonin is removed from the synaptic cleft by the highly selective serotonin transporter (SERT), which is found on the presynaptic membrane[71].

Serotonin is returned to presynaptic vesicles, where it is shielded from metabolism, after being delivered into the presynaptic neuron. The neuron's cytoplasm is where monoamine oxidase (MAO) performs its metabolic activity. In the pineal gland, serotonin can also be converted to melatonin via a different mechanism[66]. Serotonin affects the brain cells directly and indirectly through a number of processes, including: mood, sexual function, bone density, nausea, blood clotting, and bowel movement (all of which are all signs of MDD)[72].

2.4.1 Possible Genetic Linkages of Depression

2.4.2 Inflammation- Associated Genes

Multiple research suggests that MDD is more prone to inflammation, which is demonstrated by changed levels of pro- and anti-inflammatory cytokines[74]. In addition, several studies have associated MDD with a number of autoimmune conditions, including inflammatory bowel illnesses, multiple sclerosis, rheumatoid arthritis, and multiple sclerosis, indicating a very high association between inflammation and MDD[75]. Although

the relationship between depression and immunological responses is well understood, the underlying molecular pathways are largely unknown.

Cytokines, known as chemical messengers between immune cells, are the most significant critical actors in mediating depression symptoms among all immune response-related chemicals. They include numerous categories of chemicals produced by peripheral immune cells in response to stimulation from infections or malfunctioning cells, as well as Numerous studies have assessed the mRNA levels of genes related to inflammation in the peripheral blood and postmortem brain tissues of MDD patients to learn more about the function of inflammation in this disorder. For instance, a study from 2016 found that depressed patients' lymphocytes expressed pro-inflammatory cytokines and their receptors more than control subjects did, suggesting that MDD is characterised by abnormal expression of both the genes that code for pro-inflammatory cytokines and the genes that code for their membrane-bound receptors[76].

Additionally, one study found that the peripheral blood of depressive patients had increased mRNA levels of an adapter protein (ASC), which was associated with the gene for Absent In Melanoma 2 (AIM2)[77]. When a pathogen-associated molecular pattern (PAMP) or danger-associated molecular pattern (DAMP) is recognised, the inflammasome component AIM2 can activate caspase-1 via the ASC. Therefore, IL-1 and IL-18, two crucial pro-inflammatory cytokines, can be induced by the activation of caspase-1. Similarly, the Genome-Based Therapeutic Drugs for Depression (GENDEP) research revealed that non-responders to treatment for depression had greater levels of mRNA expression of genes associated to inflammation, including IL-1b, macrophage inhibiting factor (MIF), and tumour necrosis factor (TNF)[78].

In contrast, another study examined the mRNA expression of 12 genes, including some that are connected to inflammation, to study MDD in infancy and adolescence[79]. TNF, TNFR1, and IL-1b were interestingly expressed at much lower levels in the MDD group compared to healthy controls, suggesting that the modulation of the inflammatory response may be important in the pathophysiology of early-stage MDD. It has been suggested, however, that results in adults may vary from those in children[80]. In instance, characteristics that are discovered in adulthood but not childhood, such as traumatic experiences, alcohol misuse, and smoking, may have an impact on MDD in adults.

In fact, alterations in cytokine production are only one aspect of the immune system activation seen in people with MDD. In fact, it has been hypothesised that oxidative stress, an inflammatory trigger, plays a significant part in the aetiology and neuroprogression of MDD[74]. Multiple defence mechanisms work together to protect cells from reactive oxygen species (ROS) damage in physiological settings. The primary antioxidant enzymes (AOEs) include catalase (CAT), glutathione peroxidase (GPx), copper-zinc superoxide dismutase (CuZnSOD), and glutathione reductase (GLR)[74].

Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and NF-B are redox-sensitive transcriptional factors that closely regulate antioxidant protection. In support of this, a research showed that redox-sensitive transcriptional factors (Nrf2 and NF-B) and AOEs (MnSOD, CuZnSOD, and CAT) are up-regulated in the PBMC of MDD patients, indicating a pro-oxidative state. In particular, they discovered increased mRNA levels of Nrf2, Keap1, and NF-B in the cytoplasm of depressed patients' PBMC in comparison to controls[81].

The primary antioxidant enzymes (AOEs) include catalase (CAT), glutathione peroxidase (GPx), copper-zinc superoxide dismutase (CuZnSOD), and glutathione reductase (GLR)[82, 83]. Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and NF-B are redox-sensitive transcriptional factors that closely regulate antioxidant protection. In support of this, a collaborative study showed that redox-sensitive transcriptional factors (Nrf2 and NF-B) and AOEs (MnSOD, CuZnSOD, and CAT) are up-regulated in the PBMC of MDD patients, indicating a pro-oxidative state[81]. In particular, they discovered increased mRNA levels of Nrf2, Keap1, and NF-B in the cytoplasm of depressed patients' PBMC in comparison to controls.

The scientists also discovered a significant association between elevated levels of MnSOD, CuZnSOD, and CAT in individuals with MDD and Nrf2 levels, whereas elevated levels of SODs were also favourably correlated with NF-B. These results imply that the pro-inflammatory signalling seen in MDD is altered as a result of changes in anti-oxidative defence mechanisms[74].

Serotonin (5-HT), a neurotransmitter, has recently been shown to have the ability to control the immune system. The recently discovered process of serotonylation, a separate mechanism by which serotonin leads to the activation of intracellular processes, and peripheral 5-HT are both strong immune modulators that have an impact on immune cells[84].

Finally, we recently demonstrated in the Biodep study, that individuals with depression who are drug-free and treatment-resistant not only have greater levels of pro-inflammatory cytokines and chemokines, but also have increased expression of the P2X purinoceptor 7 (P2RX7)[85]. P2RX7 is widely expressed across immune system cells,

particularly microglia cells, and it plays a critical role in the activation of inflammatory processes[86]. Its expression has also been found in brain cells, where it can control how various neurotransmitters connected to MDD function[87].

Overall, these studies have demonstrated a favourable link between the upregulation of pro-inflammatory molecule expression and MDD, indicating that inflammation is one of the major mechanisms contributing to the pathophysiology and development of MDD. Additionally, these studies suggest the value of genes connected to inflammation.

2.4.3 Neuroplasticity

Currently, numerous investigations have shown that MDD also has elevated inflammatory levels and impaired neuroplasticity[88]. For instance, people with MDD have been observed to have altered synaptic and morphological plasticity[89, 90]. The intracellular mechanisms behind these abnormalities and their function in MDD have also been the subject of numerous investigations. Numerous neurotrophic/growth factors, including brain-derived neurotrophic factor (BDNF) and glial cell-line-derived neurotrophic factor (GDNF), appear to be important in neural plasticity, according to the available data[91]. In fact, BDNF plays a role in the survival, migration, proliferation, and differentiation of neurons in humans[74].

The mRNA levels of BDNF and MEK1/2, an initial activator of the MEK-ERK pathway controlled by BDNF, in the leukocytes of MDD patients and healthy controls, were one study validated this conclusion. A fascinating finding by the authors supports the involvement of BDNF and MEK1 in the pathophysiology of MDD by demonstrating reduced mRNA levels of BDNF and MEK1 in depressed patients compared to controls[92].

Hypoxia inducible factor-1 (HIF-1) is a well-known oxygen-sensitive transcriptional activator of VEGF that is induced by hypoxia, ischemia, and the activation of several different genes, including VEGF, erythropoietin (EPO), glucose transporter-1,3 (GLUT1,3), lactate dehydrogenase-A (LDHA), phosphoglycerate kinase 1 (PGK1), 6-phospho Additionally, it supports apoptosis, angiogenesis, erythropoiesis, glucose metabolism, and cell proliferation/survival[93, 94]. A research examined the levels of mRNA expression of HIF-1 (and) and its target genes (VEGF, GLUT1, PGK1, PFKFB3 and LDHA) in peripheral white blood cells of patients with MDD and bipolar disorder (BPD)[95]. The researchers discovered that MDD patients had higher expression levels of HIF-1, VEGF, PFKFB3, GLUT1, PGK1, and LDHA.

Additionally, the myelin proteolipid protein (PLP/DM20) family member glycoprotein M6a (GPM6A) of the neuronal membrane plays a significant part in the stress response in many animal models[96]. Based on this idea, an investigation proposed that changes in the expression of genes associated to stress-responsive neuroplasticity, such as those in the PLP family, may contribute to the aetiology of MDD[97]. They showed that the hippocampus of depressed suicides had significantly lower GPM6A mRNA levels. In contrast, GPM6B was down-regulated but not PLP1. All of these data imply that significant modifications in the neural connections resulting in aberrant behaviours could be caused by changes in the balance between mRNA levels of all the examined genes. These findings indicate that decreased GPM6B expression might lead to oligodendrocyte misfunction associated with MDD.

The Transcription Factor 4 (TCF4) gene may also play a role in early neuronal differentiation, be related to memory performance, and influence the brain's immune system[98, 99] . The mRNA and protein levels of TCF4 in the blood of MDD patients and

healthy individuals were examined When compared to controls, TCF4 expression at both the mRNA and protein levels was lower in patients with MDD[100]. This finding raises the possibility that decreased TCF4 mRNA and protein levels may damage cognitive functioning, which may change how MDD develops or progresses.

Overall, the research have demonstrated that the development of cognitive impairment, which is frequently seen in MDD, might be caused by a dysregulation of neurotrophic/growth factor systems like BDNF and VEGF as well as of other genes involved in the control of neuroplasticity.

2.4.4 Neurotransmitters

Serotonin, norepinephrine, and dopamine abnormalities in the brain have long been associated with MDD, and more recently, glutamate, a different neurotransmitter, has also been connected to the disorder.

It's interesting to note that studies have shown that the activation of the a-7 nicotinic acetylcholine receptor (a7 nAChR) increases the permeability to cations, such as Ca(2+)70, which in turn facilitates the release of some neurotransmitters, such as the release of noradrenaline, serotonin, GABA, glutamate, and dopamine. Cholinergic Receptor Nicotinic Alpha 7 Subunit (CHRNA7), which is partially replicated by a chimaera gene called CHRFAM7A, codes for the A7 nAChR[74].

Based on these premises, an investigation on the expression of CHRNA7 and CHRFAM7A in a sizable cohort of schizophrenia, BPD, and MDD patients' dorsolateral prefrontal cortex. They discovered that when compared to the other groups, MDD patients had considerably higher levels of CHRNA7 expression[101]. In addition, the ratio

of CHRFAM7A/CHRNA7 levels was significantly different between the diagnostic groups, and the expression of CHRFAM7A was significantly higher in all diagnostic groups than in the healthy group especially in the MDD group. These findings point to an aberrant function of nAChRs in mental illnesses.

While the effects of dopamine, 5-HT, and norepinephrine on MDD have been studied for many years, glutamate's significance in this psychiatric condition has just lately been established. Indeed, a growing amount of evidence demonstrates that MDD and other mental diseases, such as glutamate system abnormalities, are associated with changed behaviour[102, 103]. Numerous glutamate receptor (GluR) subtypes are stimulated by glutamate, an excitatory neurotransmitter that is widely distributed throughout the brain. These include the N-methyl-D-aspartate (NMDA), 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl) propanoic acid (AMPA), kainate (KAR), and metabotropic (mGluR) receptors[104]. Four studies investigated the mRNA expression of glutamate receptors and transporters in postmortem brain tissues.

In a large cohort of postmortem subjects from three diagnostic groups MDD suicide, MDD non-suicide, and a group of controls with no history of psychiatric disorders. Another study for example, tested the idea that GluR gene expression is altered in the dorsolateral prefrontal cortex (DLPFC) in MDD. In comparison to controls, they found that the DLPFC of MDD patients expressed more of a number of GluR genes[105]. In particular, they discovered that female patients with MDD had greater expression levels of GRIN1, GRIN2A-D, GRIA2-4, GRIK1-2, GRM1, GRM4, GRM5, and GRM7 than male patients with MDD.

Finally, in all samples (male and female), when MDD suicides were compared to MDD non-suicides, GRIN2B, GRIK3 and GRM2 were expressed at higher levels in the suicide victims. GRM5 expression levels, on the other hand, were lower in male MDD patients than in male controls[105].

Additionally, a diffirent study examined the gene expression levels of glutamate receptors, NMDA, and AMPA in postmortem noradrenergic LC neurons from subjects with MDD (the majority of whom died by suicide) and matched to healthy controls because several studies suggest that the locus coeruleus (LC) plays a significant role in the origin of clinical MDD and possibly suicide[106].

The scientists discovered a highly expressed GRIN1 subunit, intermediate levels of GRIN2A, GRIN2B, and GRIN2D subunit gene expression, and lower levels of GRIN2C and GRIN3A subunit gene expression. A glycine-binding NR1 subunit in combination with at least one of the glutamate-binding NR2 or NR3 subunits make up the functioning NMDA receptor complex. Calcium is necessary for activating the PI3K and CREB cell-signaling pathways, which set the NMDA family of receptor signaling apart from the other ionotropic glutamate receptors[107]. This is true even though the NMDA receptor complex is permeable to both potassium and calcium.

The levels of expression of three glutamate-related genes (two glutamate transporters, SLC1A3 and SLC1A2, and a gene encoding glutamine synthase, GLUL), as well as a glia gene (GFAP), was previously examined in postmortem tissues from men with MDD and from matched healthy controls by the same authors[108]. In people with MDD, they discovered indications of astrocyte malfunctions in the LC region, including decreased

expression levels of SLC1A3, SLC1A2, and GFAP, as well as lower levels of GFAP protein and decreased densities of GFAP-positive astrocytes.

The research described above have all shown that a number of neurotransmitters are involved in the aetiology of MDD. In particular, they have demonstrated anomalies of the glutamate system in addition to consolidating the roles of serotonin, dopamine, and norepinephrine. In fact, these investigations have found that the glutamatergic system dysfunctions and changes in the processes governing glutamate metabolism and clearance in the brain regions mediating cognitive-emotional behaviours are related to the pathophysiology of MDD.

2.4.5 Stress-Associated Genes

The HPA axis, which serves as a conduit between cognitive and non-cognitive stresses processed in the CNS and in the peripheral endocrine response system, is the primary neuroendocrine component of this stress response[109]. Several studies have evaluated the mRNA levels of genes implicated in the stress response in individuals with MDD in order to better understand the mechanisms of the stress response. The glucocorticoid receptor (GR), which is widely established to play a critical part in facilitating the antagonistic feedback control of the HPA axis[110], has recently been the subject of multiple studies looking at the expression levels and performance of the GR in patients with MDD.

For this reason, a separate study examined the mRNA levels of stress-related genes in the PBMC of MDD patients and their matched controls, including BDNF, NR3C1 or GR, FK506 Binding Protein 5, Corticotropin Releasing Hormone Binding Protein, and Corticotropin Releasing Hormone Receptor 1 (CRHR1)[111]. The transcription factor GR, which is encoded by the gene NR3C1, can act as a regulator of other transcription factors

as well as a transcription factor that attaches to glucocorticoid responsive elements (GRE) in the promoters of genes that are glucocorticoid sensitive. While GR controls the expression of BDNF, FKBP5 co-chaperones hsp90, which controls the sensitivity of GR. The authors discovered a decrease in the expression levels of the majority of the examined.

The majority of the analysed genes, including BDNF, FKBP5, and NR3C1, had lower expression levels in MDD patients compared to controls, according to the authors. This confirms that reduced expression levels of these transcripts may cause a maladaptive response to stressful stimuli, raising the risk for MDD[74].

A similar study examined the expression levels of the genes oxytocin prepropeptide encoding gene (OXT) and oxytocin receptor (OXTR), which are involved in the glucocorticoid pathway, as well as the glucocorticoid and mineralocorticoid receptors, NR3C1 and NR3C2, respectively. They noticed that MDD patients had higher OXTR expression levels and validated that there was dysregulation in the oxytocinergic signaling, which refers to proteins in the signaling pathway such as oxytocin, oxytocin receptors, and associated regulatory elements[112].

Serine/threonine kinase (SGK1), a crucial component of the cellular response and neural processes, including adult hippocampal neurogenesis, is another significant gene implicated in the modulation of the effects of glucocorticoids on brain function.

An investigation evaluated the expression of a number of possible biomarkers in peripheral blood leukocytes to evaluate the theory that stress is connected to MDD[113]. These genes include TERT, STMN1, and p16INK4a (biomarkers of telomere dysfunction and cellular senescence), FOS and DUSP1 (involved in the cell-signaling response to

biopsychological stress), OGG1 (which catalyses the repair of oxidised 8-oxoguanine DNA base and is a sensible marker of oxidative stress), and TERT. OGG1, p16INK4a, and STMN1 gene expression were all considerably higher in MDD patients' leucocytes than in controls, suggesting a link between these transcripts' over-expression and a higher chance of developing MDD[74].

Although it has generally been demonstrated that depressive individuals exhibit altered levels of gene expression associated with stress in peripheral blood samples, some of the research previously stated also highlighted the presence of conflicting results that may be connected to the pharmacological therapy the patients were receiving.

2.4.6 Whole-Genome Transcriptome Assays

Microarrays are one example of a high-throughput technology that enables the exploration of the expression levels of the entire genome and the identification of changes in gene expression using a hypothesis-free methodology. These technologies have been utilised in numerous research over the past ten years to find variations in gene expression that are linked to MDD. Transcriptomics studies can enable the identification of new biomarkers associated with MDD that can aid in the development of novel intervention strategies and the introduction of personalised medicine, in addition to the hypothesis-driven approach, which is primarily based on the analysis of candidate genes expression levels[74].

A unique study recently investigated whether changes in gene expression in peripheral blood of patients with hepatitis C at baseline are related to the development of IFN-induced MDD later on (before IFN administration) and identified longitudinal changes in gene expression from baseline to treatment weeks (TW) 4 and TW24 after IFN treatment,

in those subjects who did or did not develop MDD[114]. Specifically, patients who eventually developed MDD and those who did not had differential expression of 73 genes at the baseline. At TW4, 592 genes, mostly IFN-responsive genes, were significantly altered in the entire group; the majority of these changes only occurred in patients who acquired MDD, with an increase in genes associated to oxidative stress, inflammation, and neuroplasticity. The same outcomes were seen at TW24.

These findings unambiguously show that IFN-induced MDD patients have increased IFN-biological sensitivity. The found transcriptomics signature could be employed as a biomarker for the early detection of people who are at high risk of developing MDD or to provide molecular targets for the development of new therapeutic approaches in MDD in addition to the IFN-treatment.Additionally, in another intriguing study, which looked into the genetic profile of micro-dissected sub-fields of postmortem hippocampus from MDD participants to provide new evidence that alteration of synaptic and glutamatergic signalling pathways contributes to the pathogenesis of MDD. The genes Synaptosome Associated Protein 25 (SNAP25), Discs Large Homolog 2 (DLG2), Microtubule-Associated Protein 1A (MAP1A), and 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid receptor subunit genes GLUR1 and GLUR3 were all significantly dysregulated, according to the authors.

In a recent study, genome-wide gene expression studies were performed on depressive patients who were prospectively classified into responders and non-responders after an 8-week trial of escitalopram therapy. The genes CHN2 and JAK2 have been identified by the authors as having higher levels of mRNA expression in the non-responders group. Particularly, JAK2 stimulates both innate and adaptive immunity, whilst CHN2 may

change hippocampus neurogenesis, suggesting that both genes may be potential candidates for predictors of the response to treatment[116].

The aforementioned studies have not only supported prior findings (such as the link between abnormalities in the immune system, stress response, neuroplasticity, and neurotransmitter pathways, and MDD), but they have also demonstrated the enormous benefit of using whole-genome transcriptome assays to pinpoint pathways and molecular mechanisms.

2.5 The SLC6A4 Gene (Solute Carrier Family 6 Member 4)

Other Names

SLC6A4 is also known as: OCD1, SERT, SERT1, 5-HTT, 5-HTTLPR, 5-HTT, hSERT and HTT[117].

2.5.1 Gene Location

With Cytogenetic location at 11.2 on chromosome 17q (long arm) contains of an exon count of 15, according to the US National Library of Medicine[117] . **(Figure 2.6)**

2.5.2 Gene Size/Genomic Content

The SLC6A4 gene spans 15 exons, is situated on the plus strand, and is 41,684 bp long (**Figure 2.7**) (according to UCSC, GRCh38/hg38; NCBI Homo sapiens Annotation Release 109)[117] .

2.5.3 General Information

The sodium-dependent serotonin reuptake protein encoded by the SLC6A4 gene transports the neurotransmitter serotonin back to the pre-synaptic end from the synaptic cleft. Its major job is to stop serotonin from working and send it to the neurotransmitter

pool for recycling. This trans-membrane protein, which is a member of the sodium: neurotransmitter symporter family, is affected by the psychomotor stimulant medications, most notably amphetamines and cocaine[117].

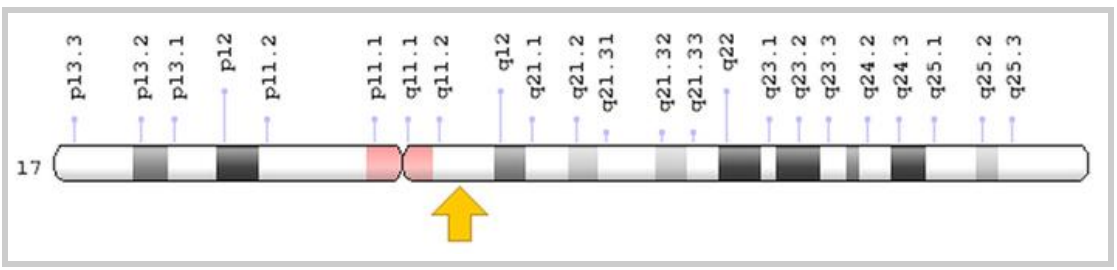


Figure 2.6: Cytogenetic Location of SLC6A4 Gene

Source[117]

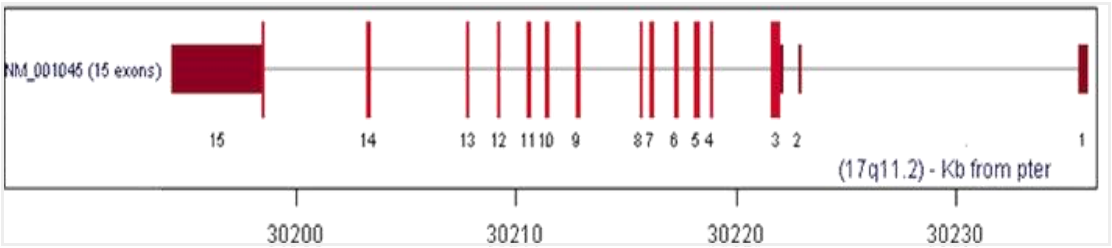
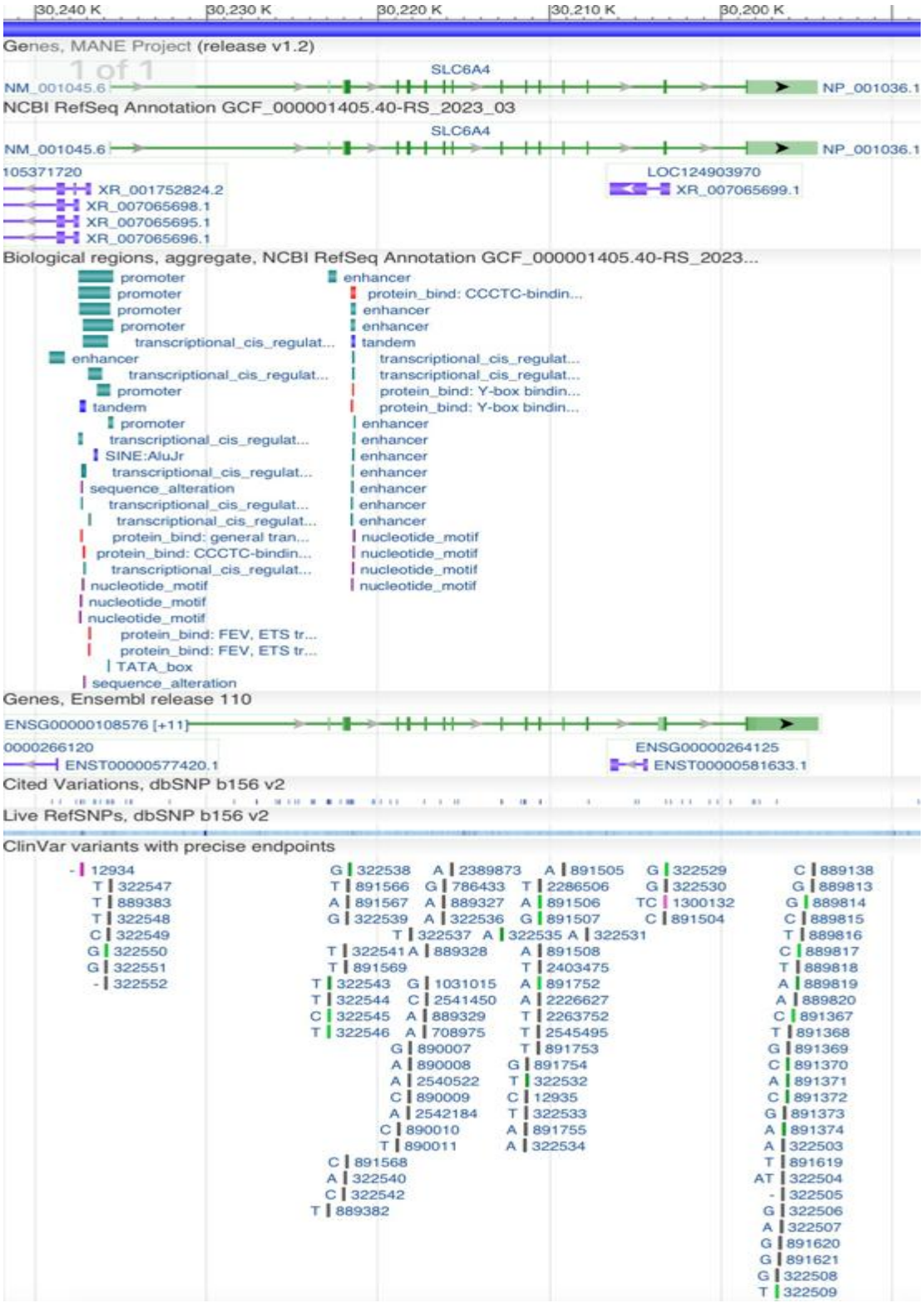


Figure 2.7: Numbers and Illustrative Sizes of Exons of Human SLC6A4

Source[118]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30,230 K
30,220 K
30,210 K
30,200 K
Genes, MANE Project (release v1.2)
1 of 1
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NM_001045.6
NP_001036.1
NCBI RefSeq Annotation GCF_000001405.40-RS_2023_03
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Biological regions, aggregate, NCBI RefSeq Annotation GCF_000001405.40-RS_2023...
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enhancer
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transcriptional_cis_regulat...
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protein_bind: CCCTC-bindin...
transcriptional_cis_regulat...
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nucleotide_motif
nucleotide_motif
protein_bind: FEV, ETS tr...
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Live RefSNPs, dbSNP b156 v2
ClinVar variants with precise endpoints
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T 322547
T 889383
T 322548
C 322549
G 322550
G 322551
- 322552
G 322538 A 2389873 A 891505 G 322529
T 891566 G 786433 T 2286506 G 322530
A 891567 A 889327 A 891506 TC 1300132
G 322539 A 322536 G 891507 C 891504
T 322537 A 322535 A 322531
T 322541 A 889328 A 891508
T 891569 T 2403475
T 322543 G 1031015 A 891752
T 322544 C 2541450 A 2226627
C 322545 A 889329 T 2263752
T 322546 A 708975 T 2545495
G 890007 T 891753
A 890008 G 891754
A 2540522 T 322532
C 890009 C 12935
A 2542184 T 322533
C 890010 A 891755
T 890011 A 322534
C 891568
A 322540
C 322542
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G 891373
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G 322508
T 322509

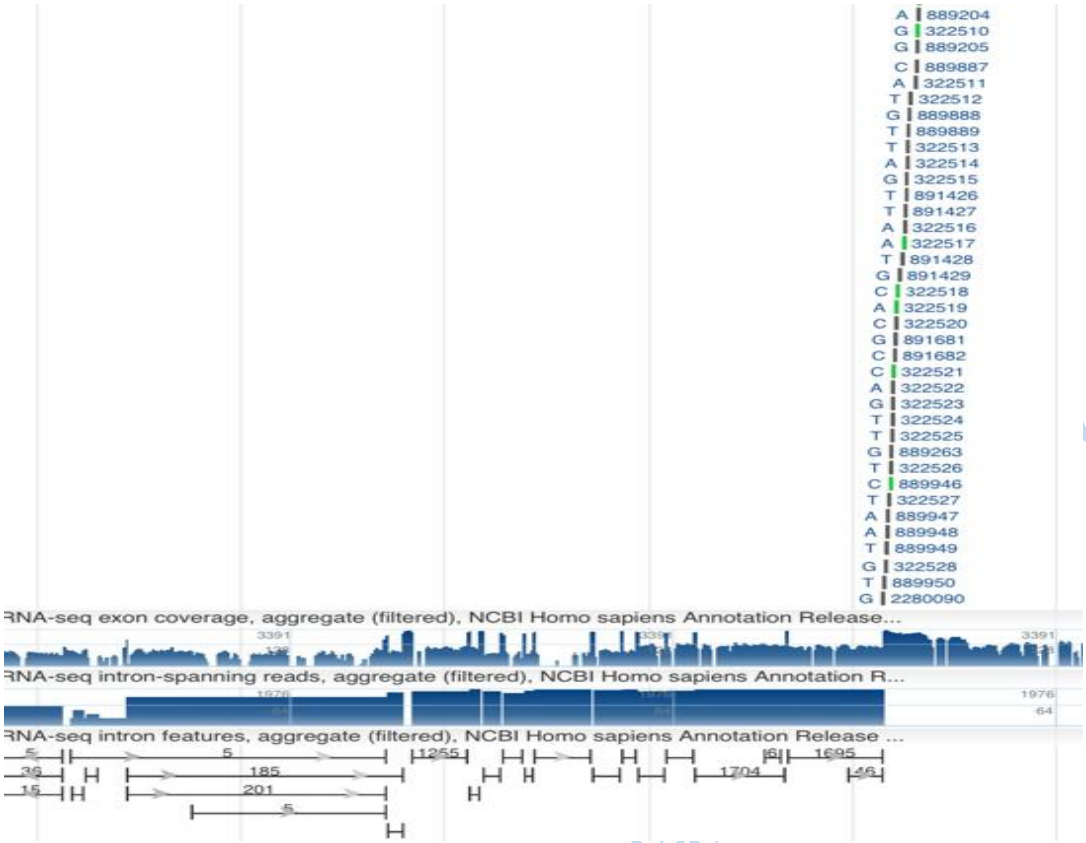

Figure 2.8: Genomic Location of SLC6A4 (Chromosome 17 - NC_000017.11 Reference GRCh38.p14 Primary Assembly)

Source[117]

2.5.4 Transcription of Human SLC6A4 Gene

Table 2.1: Transcripts of Human SLC6A4 Gene (Ensemble, GRCh38.p12).

Name	Transcript ID.	bp	Protein (aa)	Biotype
SLC6A4-201	ENST00000261707.7	6604	630 aa	Protein coding
SLC6A4-202	ENST00000394821.2	2160	618 aa	Protein coding
SLC6A4-203	ENST00000401766.6	6543	630 aa	Protein coding
SLC6A4-204	ENST00000578609.1	566	No protein	Retained intron
SLC6A4-205	ENST00000579221.5	1069	72 aa	Nonsense mediated decay

*** ID.**: Identification, **AA**: Amino Acid, bp: base pair.

Source[118]

2.5.5 Human SCL6A4 Protein

`A serotonin transporter protein (5-HTT) with a mass of 70,320 Dalton and 630 amino acids is encoded by the SLC6A4 gene **(Figure 2.9).** The protein is a member of the sodium/neurotransmitter transporter (NSS) family. Dopamine, glycine, and gamma-

aminobutyric acid (GABA) transporters are also members of the NSS family. This family's members have intracellular N and C terminal regions as well as 12 transmembrane domains. All eukaryotic NSS proteins modify the large extracellular structure (EL) between TM3 and TM4 through N-linked glycosylation, albeit the exact number of sites varies from carrier to carrier. The asparagine side chain's amide nitrogen glycan binding area and any amino acid other than proline make up the consensus sequence for N-linked glycosylation, which is N-X-S/T. Two glycosylation sites on the 5-HTT protein's EL2 contain glycan. Problems in serotonin transport at the cell surface are typically brought on by mutations in the glycosylation sites of 5-HTT and other NSS proteins[117, 118]. X-ray structure of the ts3 human serotonin transporter complexed with paroxetine (selective serotonin re-uptake inhibitor) at the central site[118].

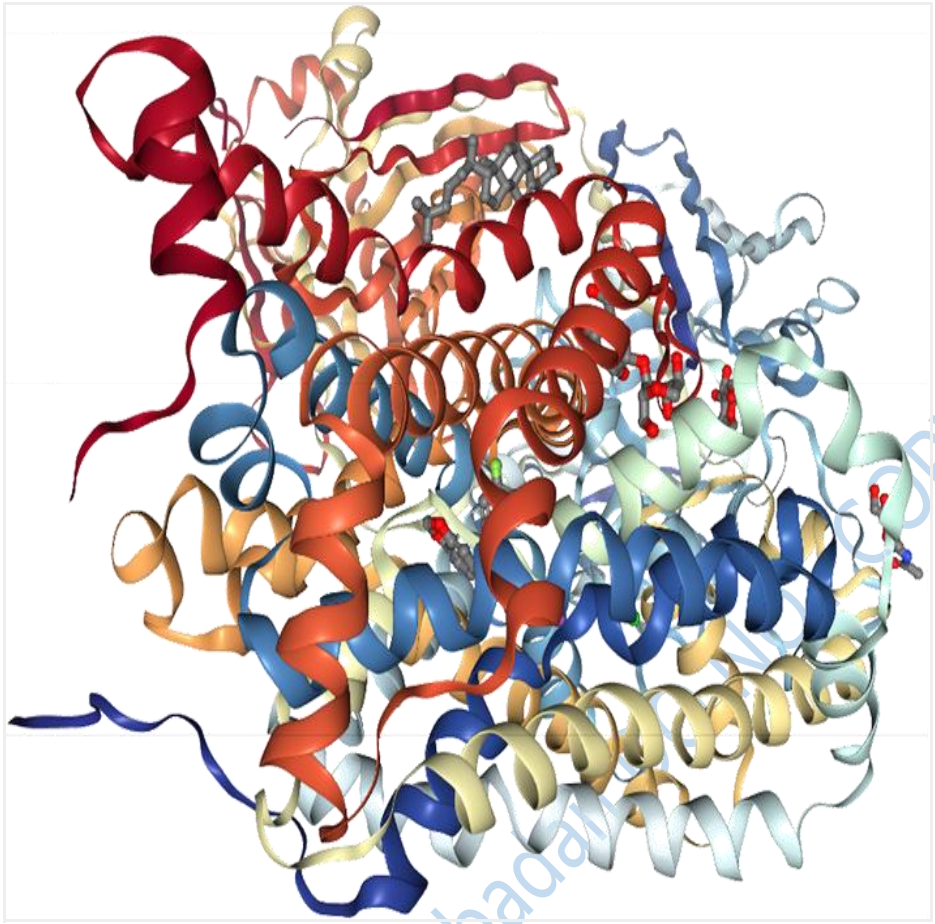

Figure 2.9: Structure of Human Solute Carrier Family 6 Member 4

Source[118]

2.5.6 Human SCL6A4 Expression

The lung, female tissues, and gastrointestinal tract are where the SLC6A4 gene is most frequently expressed (**figure 2.10**). Male, female, endocrine, muscular, and skin tissues all exhibit less of its expression[118].

2.6.7 Human SCL6A4 Localisation

SLC6A4 is present in a number of cellular compartments, including the cytosol, endosome, plasma membrane, integral parts of the postsynaptic and presynaptic membranes, endomembrane system, neuronal projection, and serotonergic synapse[118].

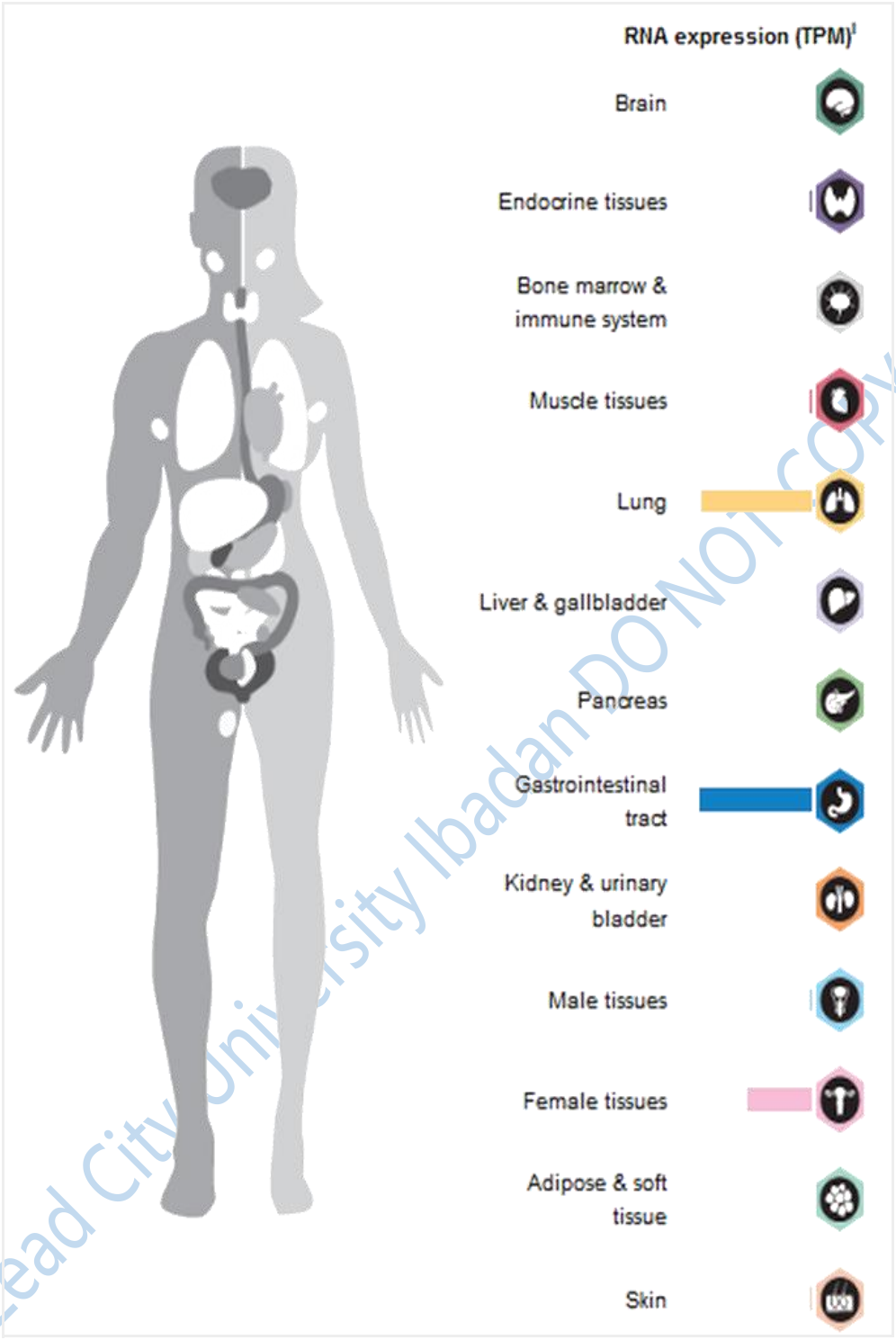

Figure 2.10: Expression Profile of SLC6A4 in Different Tissues/Organs in Humans

Source[118]

2.5.8 Other Biological Functions of SLC6A4 Gene

The levels of internal potassium as well as external sodium and chloride ions are necessary for 5-HTT protein activation (**figure 2.11**). The 5-HTT protein's ability to function is likewise reliant on the membrane potential created by sodium-potassium adenosine triphosphatase. The 5-HTT protein associates with the ions sodium, serotonin, and chloride, in that order. As a result, the 5-HTT protein enters the cell and sodium and chloride molecules that were previously linked to it are released through the membrane potential. Serotonin is released in the cell via the 5-HTT protein, which also binds a potassium ion. The potassium ion activates 5-HTT, which may be present outside of the cell. An essential neurotransmitter found in both the central and peripheral nervous systems is serotonin (5-hydroxytryptamine; 5-HT)[118].

After 5-HT is released into brain synapses, it is efficiently removed from the synaptic space by the high-affinity serotonin transporter SLC6A4 (also known as 5-hydroxytryptamine transporter, 5-HTT, or SERT), which is positioned in presynaptic neuronal membranes. Thus, 5-HTT stops the synaptic activity of 5-HT and reintroduces it to the neurotransmitter pool for future use. In this way, 5-HTT plays a crucial part in serotonin retrieval and serotonergic function execution.

A number of G-protein-linked receptors and protein kinase-associated pathways, such as protein kinase C (PKC), PRKG1 (protein kinase G, PKG), and p38 mitogen-activated protein kinase (MAPK), rapidly control the 5-HTT activity. The 5-HTT protein is sensitive to extracellular 5-HT and is phosphorylated and down-regulated by PKC, which both serve regulatory roles in the movement of 5-HT[118].

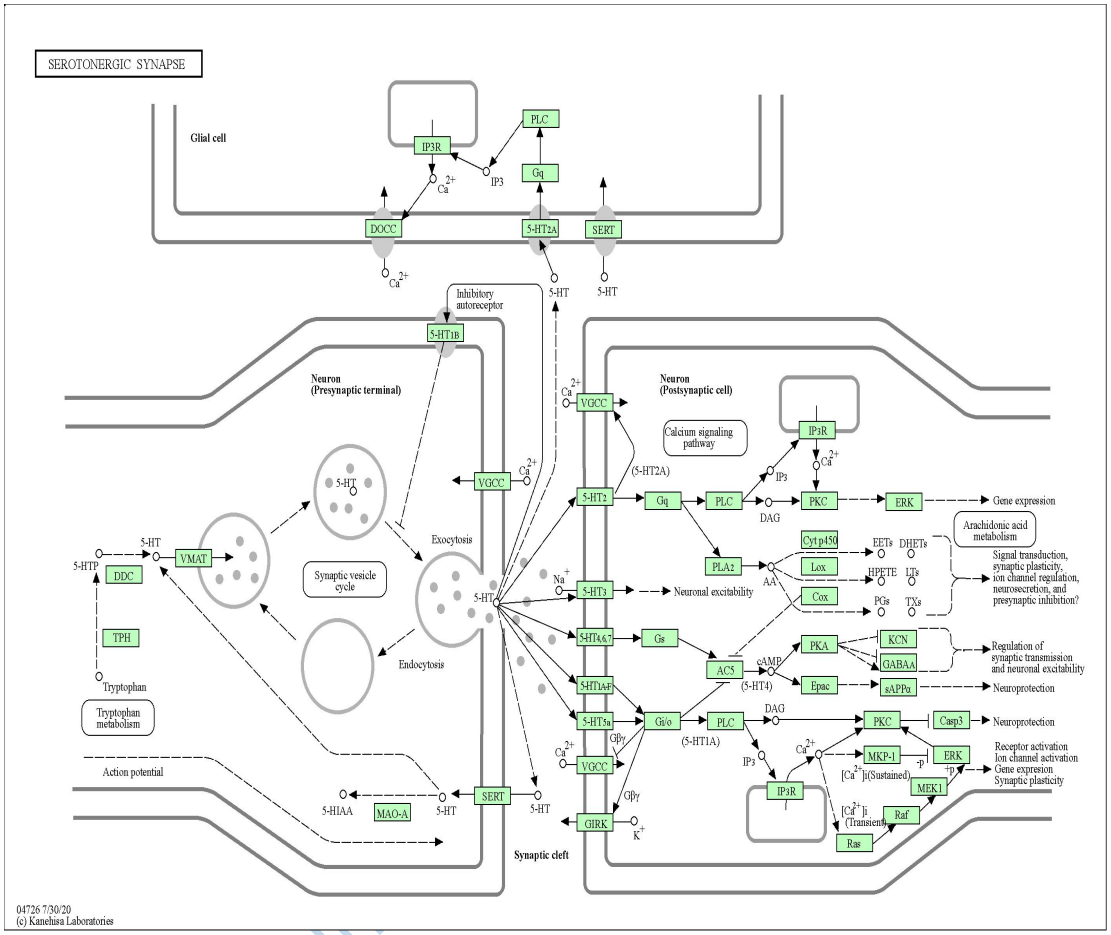

Figure 2.11: Serotonergic Synapse Pathway Map

Source[118]

5HT binds to receptors (**figure 2.11**) , which have been classified into 7 subfamilies based on common structural features and signalling pathways, after being released from presynaptic axonal

terminals. These families include the G-protein-coupled 5-HT1 (Gi/Go-coupled), 5-HT2 (Gq-coupled), 5-HT4/6/7 (Gs-coupled), and 5-HT5 receptors, as well as the ionotropic 5-HT3 receptors. It is believed that the autoreceptors that inhibit excessive 5-HT release are localised at the presynaptic site, or 5-HT1B receptors. Reuptake of 5-HT into neurons by transporters stops its activities, which triggers monoamine oxidase catabolism[118].

2.5.9 Mutations of the SLC6A4 Gene

Most frequently occurring mutations are found in the 5-HTTLPR region of the SLC6A4 gene's promoter. **Table 2.2** is a table of several mutations, their locations, proteins and observable diseases associated with[117, 118].

Table 2.2: Mutations of the SLC6A4 Gene.

S/N	Location	Mutation	Protein	Observed Phenotype
1	Exon 2	c.10A>G	p.T4A	Increased 5-HT transport

				activity
2	Exon2	c.167G>C	p.G56A	Autism
3	chr17:30218213	c.603G>C	p.K201N	Enhanced transporter activity
4	Exon 4	c.643G>A	p.E215K	MAPK nonresponsiveness
5	Exon 6	c.878C>T	p.S293F	Increased 5-HT transport activity
6	Exon 7	c.1016C>T	p.P339L	Lowered uptake activity
7	Exon 8	c.1084C>A	p.L362M	Increased 5-HT transport activity
8	Exon 9	c.1273A>C	p.I425L	Autism, association with
9	Exon 9	c.1273A>G	p.I425V	Obsessive-compulsive disorder,susceptibility, association
10	Exon 10	c.1393T>C	p.F465L	Increased rigid-compulsive behavior in autism,

Source[117,118]

S/N	Location	Mutation	Protein	Observed Phenotype
11	Exon 12	c.1648C>G	p.L550V	Increased rigid-

				compulsive behaviour in autism
12	Exon13	c.1815A>C	p.K605N	MAPK non-responsiveness
13	Exon 14	c.1861C>T	p.P621S	MAPK non-responsiveness
14	chr17:30216371	c.838-155G>A	NA	Autism
15	chr17:30237328	c.-1936G>A	NA	Obsessive-compulsive disorder
16	chr17:30237152	c.-1760T>C	NA	Obsessive-compulsive disorder,
17	chr17:30222880	c.-185A>C	NA	Major depressive disorder
18	chr17:30197993	c.463T>G	NA	Increased expression
19	chr17:30197786	c.670T>G	Na	Panic disorder

Source[117, 118]

S/N	Location	Mutation	Protein	Observed Phenotype
20	Promoter	c.1212-1255 del TGCAGCC	NA	Anxiety related traits
21	Location	Repetition Sequence	Number Repetitions	Of Observed Disease/Phenotype
22	Intron 2	(GGCTGYGACCYRG RRTG)n	10-12	Unipolar disorder
23	Intron 2	(GGCTGYGACCYRG RRTG)n	12	Pulmonary arterial hypertension

Source[117, 118]

2.6 5HTTLPR and the SNPs

The 5-HTTLPR insertion/deletion in the gene's promoter region has received the most attention. There are less transporters on the presynaptic neuron in people who have the deletion (also known as the "short" or "S" allele) as compared to people who have the insertion (also known as the "long" or "L" allele) (**Figure 2.12**).

The SLC6A4 gene has a many polymorphisms that are linked with many conditions, some of which are associated with depression[117]. Listed bellow are a few:

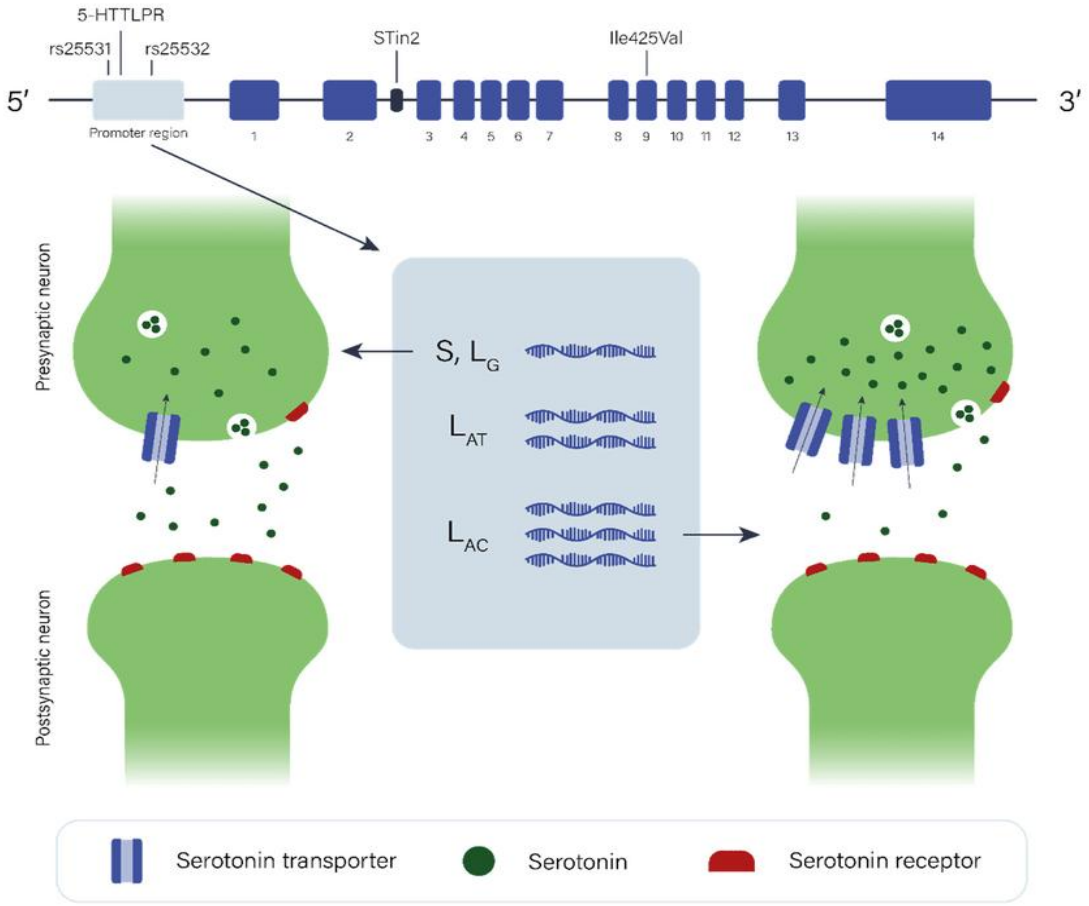

Figure 2.12. Serotonergic Neurotransmission and SLC6A4

Source[119]

The top portion of the picture shows the SLC6A4 gene, exon counts, and the polymorphisms 5-HTTLPR, rs25531, rs25532, STin2, and Ile425Val. various 5-HTTLPR/rs25531/rs25532 SLC6A4 mRNA expression (bottom middle) is probably

influenced by three-locus haplotypes. The S and L G haplotypes are thought to have low levels of SLC6A4 mRNA expression (bottom left), but the L AC haplotype is thought to have high levels of SLC6A4, which results in enhanced SERT in the presynaptic neuron and increased serotonin clearance from the synaptic cleft (bottom right). 5-HTTLPR stands for SERT-linked polymorphic region; S stands for 5-HTTLPR short allele; L G stands for 5-HTTLPR long allele and minor allele of rs25531; L AT and L AC stand for 5-HTTLPR/rs25531/rs25532 three-locus haplotypes that vary in rs25532 alleles[119].

Table 2.3. SNPS and their Associated Conditions.

S/N	SNP	Location	Allele Change	Associated Conditions
1	**rs8076005**	chr17:30220192	G>A / G>C	Hot flashes

		(GRCh38.p14	Intron Variant	(Endocrinology & Metabolism) Depression, IL-6.
2	rs25528	chr17:30222960 (GRCh38.p14)	H>A / G>T Intron Variant	Depression
3	rs2020936	chr17:30223796 (GRCh38.p14)	G>A / G>C / G>T Intron Variant	Parkinson's disease.
4	rs12150214	chr17:30223870 (GRCh38.p14)	C>A / C>G / C>T Intron Variant	Major Depression Disorder
5	**rs6354**	chr17:30222880 (GRCh38.p14)	>A / G>C / G>T 5 Prime UTR Variant	Major Depression Disorder Parkinson's disease. ADHD Alzheimer's disease. Chronic Periodontitis
6	rs11080122	chr17:30220317 (GRCh38.p14	T>A / T>C / T>G Intron Variant	Stress
7	rs25531	chr17:30237328 (GRCh38.p14	U>C / T>G Intron Variant	Major Depression Disorder

Source [119]

2.6.1 SLC6A4 Phenotypes, Serotonin Levels and Depression

Population genetic research has demonstrated that the S allele reduces the transcriptional efficiency of the 5HTT promoter gene, lowering serotonin transporter affinity and uptake.

In light of this, there may be a higher chance of being susceptible to psychiatric disorders like MDD as a result of this genetic change, while the L allele does the opposite in terms of transcriptional efficiency and increases serotonin reuptake and acts as a sort of protective allele against a myriad of psychiatric disorders and many[120].

Researchers have grouped the variations to make understanding easier because the 5HTTLPR-rs25531 variant can be present in the 5HTTLPR-VNTR variant. The L'L' group stands for LA/LA, the L'S' group for a long allele and an efficient transcriptional allele (La/Lg or La/Sa), and the S'S' group for two alleles with low transcriptional efficiency (Sa/Sa, Lg/Sa, or Lg/Lg). The variants were categorised as S'S' (Lg/Lg + Lg/S + S/S), L'S' (La/Lg + Lg/S), and L'L' (La/La) by a research team and observed that 5 HTTLPR-rs25531 will be the SL, and LS for 5HTTLPR-VNTR to facilitate understanding.

Currently, considerable research has been done on the polymorphisms of 5-HTTLPR and 5-HTT-VNTR related to emotion, mentality, personality disorders, and personality traits[122]. As PTSD can be treated with selective serotonin reuptake inhibitors (SSRIs), which target the serotonin transporter (SLC6A4), several genetic research on the disorder have also focused on the serotonin system. A gene-linked polymorphic region in the 5-HTT promoter region, the 5-HTTLPR variant of the SLC6A4 gene controls the levels of gene expression[123]. This variance could affect how the body reacts to SSRI therapy. More

than 300 neurological and psychiatric illnesses have been the subject of 5-HTTLPR research up to this point, including PTSD, major depressive disorder, and Alzheimer's disease[123]. Parallel to this, similar investigations have discovered that the polymorphism of the 5-HTTLPR gene is a substantial contributor to the cognitive impairment of Alzheimer's patients, and major depressive disorder also significantly impairs the cognitive control behaviour identified in the 5-HT 1A gene[124].

The L allele is substantially more common in Western societies than in Asian communities, with the S allele generally present in 42% of Caucasians and 79% of Asians[120]. The S allele of the 5HTTLPR-VNTR mutation may be associated with an elevated risk of depression by negatively influencing serotonin reuptake rate because of its low expression, according to a Caucasian population study with 46 MDD patients ages 19 to 58 years. An Asian population study found that more than 60% of the MDD samples had the current allele, and another study investigation in Brazil found that the S allele was the most prevalent in their sample[125] .

As a result, the S allele may be linked to a higher incidence of depression, possibly as a result of its negative effects on serotonin absorption rate. The S allele, however, was not associated with an increased risk of developing depression[120].

In heterozygous individuals, the combination of the short and long alleles of the 5HTTLPR-VNTR has a statistically higher likelihood of MDD development than in

homozygous persons (OR = 1.42, p = 0.02), Turkish and Mexican populations showed comparable outcomes[121,126].

In addition to other gender- and age-related changes, a study showed that a lifetime of MDD was linked to persistent volume reductions in the deep nuclei, insular, thalamus, ventral diencephalon, pallidum, and nucleus accumbens as well as with a broader pericalcarine region in both men and women. In terms of the 5HTTLPR genotype, the same study found no discernible volumetric differences between the groups with and without lifetime MDD according to 5HTTLPR, but discovered that participants with lifetime MDD and LL genotypes had smaller thalamus volumes and participants with lifetime MDD and SL genotypes had larger pericalcarine and lingual volumes[126]. Though these findings should be interpreted with caution, the S allele is perceived as a risk factor for mental and physical distress in younger populations, whereas in older adults, the LL genotype appears to be a risk factor for those who are highly exposed to chronic disorders and severe stressors. In summary, the Nervous System in MDD patients may be affected by the 5HTTLPR genetic mutation, whether it is VNTR or rs25531.

Studies have not been able to establish a statistical link between childhood trauma and the 5HTTLPR polymorphism, A particular Turkish, likewise did not establish a link between childhood trauma and the 5HTTLPR-VNTR variant in MDD patients residing in Turkey (p = 0.28)[127]. S allele carriers demonstrated a higher likelihood of developing depressive

symptoms in response to childhood maltreatment than people with the L allele, which is consistent with findings from the literature[120]. However, this interaction was not seen. Additionally, it is believed that the degree of hardship experienced as a child/adolescent affects the likelihood of developing depression. This theory is consistent with epidemiological research that show depression is twice as likely to develop in adults who experienced abuse as children[127].

Another comorbidity linked to MDD is suicide, which raises serious public health concerns. A Mexican research studied 200 Mexican teenagers (aged 11 to 18) who had attempted suicide during the six months prior to the survey and had depression to ascertain the relationship between the 5HTTLPR-VNTR variation and the suicide attempt and its associated diseases. Their most popular suicide methods were non-violent ones like drug overdose. The participants did, however, also mention hanging and chopping[128]. The findings of this genetic analysis of the investigation support the hypothesis that the S allele, or the SS genotype, is associated with suicide.

Of 64% of the family members who were evaluated, in an epigenetic study with 203 research participants conducted in the United States had a high risk for depression. These high-risk participants also had greater levels of impulsivity ($p = 0.0013$), aggression ($p = 0.017$), and neuroticism ($p = 0.013$) in addition to two copies of the S allele in the 5HTTLPR-rs2553 polymorphism. In other words, individuals with a high frequency of the SS genotype acted impulsively, without considering or analysing the circumstance, are contrarian and alienated, and prone to suffer negative outcomes in typical life situations (envy, anger)[120].

While a Brazilian study found that roughly 70% of the studied children (n = 40) who lived with depressed mothers (n = 40) displayed some sort of psychiatric disorder, whether it was depression, generalised anxiety disorder, or Attention Deficit Hyperactivity Disorder (ADHD), the study was not conducted in the United States. Furthermore, half of the 40 investigated depressive mothers had a history of depression in their families. There was no connection between the S allele and the pattern of depression occurrence between mother and child (p 0.999) in a study looking at the association of the 5HTTLPR polymorphism and the CpG (5mC) DNA methylation levels of the AluJb repeat element in the SLC6A4 promoter region (5HTTLPR) of a mother-child exposed to maternal depression[125].

Experimental data have shown a connection between the genetic mutation 5HTTLPR and the medications taken by MDD patients. Studies on several populations have revealed a high association between the L allele and selective serotonin reuptake inhibitors (SSRIs), as well as the S allele and SSRIs[129].

However, an Asian team found a statistically significant correlation between the gene polymorphism and the pharmaceutical treatment (lowering of the HAM-D score) in a Thai investigation. There was no decrease in the HAM-D score in depressive patients with the LL genotype (p = 0.042) despite treatment with Duloxetine or Paroxetine, both SSRIs. Asian studies have also revealed a significant S allele frequency in individuals who have experienced a positive treatment response [129]. It's intriguing to see the gender disparity in therapeutic response because women appeared to respond to treatment better than men[129].

This gap might be caused by biological causes, including menopause, or just the fact that women are more likely to follow their treatment regimens[129]. However, considering that more than 70% of the investigated population was female, a proportion that was comparable across studies[121], this finding has to be confirmed in additional research.

2.6.2 Sex Difference In Depression

Depression risk varies depending on gender. Given that sex-based disparities in lifetime rates of MDD are a distinguishing feature of the condition, this is noteworthy. For instance, rates of depression are comparable and relatively low before the pubertal transition, with about 3% of kids matching the criteria for MDD over the previous year. However, during puberty, rates of MDD nearly double for both boys and girls which is startling but significant sex differences also appear, making teenage girls at least twice as likely to experience depression as boys. A number of processes, including stress generation, detrimental cognitive biases, and altered HPA axis responsiveness to stress, have been postulated to explain this dramatically increased risk for MDD in females compared to boys. Although none of these are capable of creating susceptibility to depression[130].

The difference in the levels of the sex hormones testosterone, oestrogen, and progesterone especially throughout the reproductive years is a significant biological difference between the sexes.

As a result, it appears that the levels of absolute sex hormones in sad participants are not different from those in the control group. However, there are signs that alterations in sex hormones, particularly progesterone and oestrogen, may be followed by worsening depression symptoms over time[131].

A study that employed a double-blind, placebo-controlled design, which showed that the contraceptive pill, but not the control, had appreciable detrimental impacts on the treatment group's sleep and mood[132]. About half of the women, who have PMS symptoms, which include exhaustion and a sad mood in the week before the start of menstruation. These symptoms, which include severe anhedonia, and/or anxiety in 13–18% of women, meet the requirements for "premenstrual dysphoric disorder," or PMDD, as defined by the DSM-5[133]. Since PMDD is characterised by fatigue, poor sleep quality may also be a factor. Additionally, although these findings are not consistently replicated, some studies also found links between the use of oral hormonal contraceptives (OC) and depressed symptoms, antidepressant use, and the risk of suicide[134].

It is not yet known whether these side effects are brought on by exogenous or endogenous hormone alterations because hormonal contraceptives lower endogenous hormones by injecting exogenous hormones. Sleeping issues, often known as "trouble sleeping" and "sleeping problems", are a typical complaint and a melancholy mood is frequently experienced during the menopausal transition, when sex hormones dramatically fluctuate and eventually subside. In certain circumstances, women going through menopause might get their depression symptoms and sleep issues under control by taking oestrogen. Overall, we may claim that an increase in depressive symptoms and sleep issues frequently tend to coincide with relative changes in oestrogen or progesterone levels[133].

2.6.3. Sex Difference, Inflammation and Depression

Despite strong evidence of dimorphism, research that examined the role of sex in the complicated interaction between CM, MD, and inflammation were conducted. Starting at the beginning of adolescence, it has been repeatedly discovered that girls have higher rates of MD than boys do[130]. One possible explanation for this difference is that females

typically have a stronger pro-inflammatory response to immunological stress. However, both in men and women, a higher inflammatory response has not consistently been linked to more depressed symptoms, with some theorising that sex hormones may play a role[130]. Sex hormones can attach to immune cells and have diverse pro- and anti-inflammatory effects[130].

According to a study, low-grade inflammation could be a pathophysiological explanation for the common, challenging-to-treat condition of depression, which frequently coexists with somatic disorders[135]. When compared to healthy controls, inflammatory markers such C-reactive protein (CRP), interleukin-6 (IL-6), and tumour necrosis factor alpha (TNF-) are higher in depressed patients[136] and may be related to differences in how well they respond to antidepressant medication. Prior research was constrained by a number of factors, primarily cross-sectional designs[136], short follow-up using composite measures of depression, and only measuring one or a small number of inflammatory markers once[136]. Inflammatory markers may change depending on a variety of factors.

Therefore, recent personalized-medicine methods in the field of "psychoimmunology" have emphasised the significance of taking changes in a number of inflammatory markers over time into account and studying particular symptom dimensions, especially the neurovegetative symptoms[137, 138]. Second, meta-analyses have discovered that the inflammatory markers CRP, IL-4, IL-6, IL-10, IL-1, TNF-, and Csingle bondC motif ligand 2 chemokine (CCL-2) decrease with short-term antidepressant treatment. Trials varied widely, nevertheless, with the particular limitations that the majority only tracked patients for two time points while measuring changes in a small number of inflammatory markers, and the majority of studies lacked data on crucial cofounders like smoking and body mass index (BMI)[139].

Consequently, investigations with multiple assessments of numerous inflammatory markers during the course of extended antidepressant trials along with comprehensive information on significant cofounders are required. According to the patient's immunological profile, these studies may aid in identifying specific indicators that could direct the development of more individualised treatment plans[139].

According to one study, it has been discovered that the four main types of estrogen; estrone, 17beta-estradiol, estriol, and estetrol have a U-shaped effect of action, with low concentrations being linked to pro-inflammatory functions and low concentrations being linked to anti-inflammatory functions while progesterone and testosterone have mostly been described as anti-inflammatory[130].

Oestrogen action has been connected to the increased synthesis of pro-inflammatory mediators in response to a challenge from females. Consequently, it has been shown that physiological oestrogen concentrations increase the production of IL-6, IL-1, and TNF in response to a stimulus of human monocytes and murine macrophage[140].

On the other hand, it has also been demonstrated that eliminating endogenous oestrogen lowers immune cells' pro-inflammatory response. The reason why females typically exhibit a better sepsis outcome than males might be due to this larger acute inflammatory response[141].

Interleukin 6 (IL-6) is a distinct cytokine that has frequently been shown to be higher in depressed individuals. There is proof that IL-6 may have a role in some people's sensitivity to depression or stress. According to a longitudinal study conducted on

humans, people who had greater levels of circulating IL-6 as children were more likely to experience depression or psychosis in their late adolescent[140].

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Chapter Three

Methodology

3.1 Study Design and Sample Size

After ethical clerance was obtained (see appendix 1) for this study. The sample population included both clinically diagnosed Major Depressive Disorder and a healthy population. Both samples for individuals with depressive disorder (test) and health individuals (control) were obtained from the Psychiatric ward, National Hospital, Garki, Abuja; Neuropsychiatric Hospital, Rumigbo, Port Harchourt, Rivers State and Yaba Psychiatric Hospital, Yaba, Lagos.

Samples were collected from Abuja, Lagos, and Port Harcourt—three major urban centers in Nigeria—due to their diverse and representative populations. These cities serve as key geopolitical and socio-economic hubs, attracting individuals from various ethnic,

cultural, and linguistic backgrounds. As a result, they provide a heterogeneous population ideal for genetic studies, increasing the generalizability of findings across the broader Nigerian populace.

Additionally, these locations have well-established tertiary health institutions and psychiatric services, facilitating access to diagnosed cases of Major Depressive Disorder (MDD) and appropriate clinical support. The availability of infrastructure for ethical sample handling, storage, and transport further supported the selection of these sites for robust and reliable data collection.

Control group samples were collected from individuals who had no history or symptoms of clinically diagnosed depressive disorder from the above listed institutions. Blood samples were obtained from all participants of the study.

Sample size at 3.9% prevalence was calculated, Using OpenEpi, sample size (proportion) at 95% was calculated as 78 patients, with a control of 78 healthy individuals which came up to 156 samples. Inclusion of attrition rate of 10% for lost-to-follow-up, was added to total 172 samples[1, 2].

3.1.1. Recruitment and Sampling

Participants were recruited serially. Two questionnaires (The Hamilton Depression Rating Scale (HDRS) questionnaire and a socio-economic questionnaire) (see appendix 2 and 3 respectively). The Hamilton Depression Rating Scale was originally unstructured for clinical interviews[3]. The 17-question scale is now being clinically administered to determine degree of severity for MDD. The Hamilton Depression Rating Scale (HDRS) was selected for this study because of its broad clinical use, strong psychometric validity,

and capacity to assess the severity of depressive symptoms across various domains. As a clinician-rated instrument, it provides standardized evaluations and minimizes subjective bias—an essential feature for accurately linking clinical presentations with genetic and biochemical markers. Both will be were issued at the point of sample collection. All participants (test and control) were provided a questionnaire to give personal, social, habitual and economical information.

Current Depressive symptoms measured with the Hamilton Depression Rating Scale (HDRS) being the second questionnaire issued (this was issued by the health care official to all intending participants diagnosed with MDD).

Age and gender stratification were taken into consideration and in-order to provide a proper overview of the age and gender disparities in past literature and its implications in the severity of depression as discussed in the literature[4]. Data grouping is as follows: women were categorised into twenty and below, while men were grouped into 40 and below. This grouping was done in line with sample identification numbers in order to maintain proper tracking of samples during storage, transportation, laboratory investigation and data analysis.

3.2 Ethical Approval, Consent, Confidentiality and Withdrawal

Ethical clearance was obtained from Federal Ministry of Health (with approval number: NHREC/01/01/2007-01/09/2024) Lead City University and Respective institutions where Sample collection and Sample Analysis took place (Psychiatric ward, National Hospital, Garki, Abuja; Neuropsychiatric Hospital, Rumigbo, Port Harchourt, Rivers State and Yaba Psychiatric Hospital, Yaba, Lagos). All ethical approval documents are provided (see appendix 1)

Depressive patients and non-depressed individuals (control group) recruited for this study were over 18 years old, and as such consent was obtained before sample collection and testing (see appendix 4), each patients' samples and their respective questionnaire forms were designated a unique identification number in order to uphold confidentiality of information. Individuals who wished to no longer participate in the study at any point were allowed to drop out and have their samples removed from the study population (a 10% attrition rate was added to the sample size to offset such instances).

NDAs (see appendix 5)were constructed for the peculiarity of this study and issued to the to all members of the research team (Doctors, Laboratory Assistants, Data Analysts, and any other personnel) involved in the direct handling of study population information. Master sheets that contain patients' information will be compiled singularly by the primary investigator, recorded on encrypted files and stored on a password-ed system, and only accessible to the the supervisory and primary investigators

3.3 Inclusion/Exclusion Criteria

Participants included men and women between the ages of 18-60 years. Only participants that met the HDRS symptoms were admitted into the MDD population.

The following individuals will not be included into this study:

Individuals with other:

1. mood disorders;
2. with recent and apparent loss, and
3. with head trauma.

3.4 Sample Type, Collection and Processing

Sites used their routine method to diagnose patients with depression. Patients at each site were approached and informed about the study. Those who gave informed consent were recruited serially at each site and filled questionnaire at recruitment to provide personal, social, habitual and economical information.

Blood Samples (10 mls) were taken via venipuncture and collected in EDTA bottles. Unique identification numbers were issued that tally with the both questionnaire forms. Samples were stored at -20°C. Repeated freezing and thawing was avoided.The samples will be collected at the various hospitals (Psychiatric ward, National Hospital, Garki, Abuja; Neuropsychiatric Hospital, Rumigbo, Port Harchourt, Rivers State and Yaba Psychiatric Hospital, Yaba, Lagos.) and stored at the institutes' provided -20°C cooling systems.

Two questionnaires were administered at recruitment and the point of sample collection. The clinicians on the study team administered the Hamilton Depression Rating Scale (HDRS) to measure the current depressive symptoms. Participants' cases were categorised into normal, moderate and severe depression. Sex-matched control patients were recruited from general ward at National Hospital, Abuja.

After which they were transferred to the Center of Human Virology and Genomics, Microbiology Department, Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, where the genotyping was done. Hormonal testing was carried out at the Chemo-pathology laboratory. Data Analysis will be carried at thereafter using appropriate data analysis methods.

The 10 millilitres of blood was then divided into aliquots of, 2ml of blood collected in EDTA bottles will be sent for qPCR testing for detection of the SLC6A4 CNV and SNPs.

The remaining 8mls of blood upon separation will produce an average of 4 ml of serum was used for hormonal and pro-inflammatory (IL-6) testing, which included; serum serotonin testing used ELISA kits; serum/plasma IL-6 levels were measured using ELISA kits; Testosterone, Estrogen and Progesterone concentrations determined using Chemiluminescent Microparticle Immunoassay (CLIA Microparticles) kits. Samples were stored at -20°C and a cold chain was maintained during transportation and at the various sites.

3.5 Data Collection, Management and Statistical Analysis

1. Participants were categorised based on their HDRS scores in severity of depression. Data from questionnaires was used as descriptive information.
2. Genomic data provided from the qPCR was observed using Design and Analysis Software 2.0 (DA 2.0)
3. Descriptive statistics was computed and represented in Frequency/Graph tables.
4. Hardy Weinberg Equilibrium was used to check for genotypic predisposition, allelic frequency and phenotypic distribution.
5. Data obtained was analysed using EpiInfo (Center for Disease Control and Prevention, Epi Info Version 7.2.4.0, 2020)[5].
6. Student's t test and Chi square was used to check the differences between two groups.
7. ANOVA was used to find significance between the mean and standard deviation of study groups.
8. Kruskal Wallis was used test the differences between study groups.
9. Pearson's correlation was to find the correlation between groups.
10. Statistical significance (p) was s*et* at < 0.05 and 95% confidence interval.

3.6 Anticipated Issues and Expected Outcomes

Anticipated Issues

These are likely obstacles that were faced during the course of this research work;

1. Unwillingness of Individuals to Participate in the Study: given the subject matter (participants were less likely to provide their blood samples for the study analysis). Participants were provided an informed consent form which will contain explanations of the study matter, process and importance to them and community. All participants were above 18 to give them the advantage of being less likely coerced and there was also an introduction of principle investigators within the said form to enable them share any reservations, as contact addresses are provided as well. Adults of Legal age were enrolled into the study to make sure "consent" could be taken.
2. Maintaining of Sample Integrity: all individuals responsible for the collection of the samples are seasoned professionals. The samples of the MDD population were collected by only licensed psychiatric doctors and the samples labelled and processed (Centrifugation) at the site to preserve the needed aliquot by experienced laboratory scientists. Provisions for storage in freezers to stop denaturation were made.
3. Storage and Transportation of Samples: Samples in storage and during transportation were be kept at -20°Cstrictly, as repeated freezing and thawing of samples could have impacted on the integrity of the samples. Proper handling techniques: such as using airport delivery systems with ice boxes provided all throughout the various sites of collections.
4. Cost of the Study: The research proceedings and handling of samples maybe have been very expensive and cumbersome as there a number of different analytical process to be carried out. However, the primary investigator was highly dedicated and set to provide all resources required within the duration of the research.

5. The Identities of the Study Participants: NDAs were constructed for the peculiarity of this study and issued to the to the research team all contract personnel that may have come in contact with patient information while working with working on the research team (Doctors, Laboratory Assistants, Data Analysts, and any other personnel).
6. Integrity of Results: the data analysis was issued to two independent analysts to produce results and compare findings, and reproduced multiple times to ascertain validity.

Expected Outcome

Listed below are some of the outcomes;

1. Serum serotonin levels were expected to be raised in depressive patients.
2. SLC6A4 short alleles were expected to be more predominant in the sample population as reported in other research findings.
3. A possible increase in some cases of IL-6 levels to hint at inflammation in the depressive population.
4. A possible correlation between hormones and pro-inflammatory hormones.

3.7 Project Plan and Implementation

Project planning was followed according to the information and the periods for each analysis/stage of the work will be followed so as to limit extensions.

3.7.1 Dissemination of Study Findings

All results and findings will be published in prospective journals and publishing houses. Important findings will also be shared with the community through campaigns on and off social media platforms for sensitization in genetic testing and alternative treatment options for individuals.

3.8 Monitoring and Evaluation Plan

Laboratory work was monitored and all observations and findings recorded to provide a well documented work, so as to keep track of all trends present within the sample population.

All proceedings followed ethical and good laboratory practice.

3.9 Responsibility/Litigation

No study participants information will be made public any member of the research team within the provisions of the NDA documents. Participants who no longer wished to engage in the study were allowed to withdraw their information and specimens at any stage of this study. The research team was available and ready to supply data and information of study processes upon request. This study will be carried out according to the principles of the "Helsinki Declaration"[6].

3.10 Laboratory Procedures and Analysis

- Whole blood genomic DNA extraction was carried out using the TIALONG automated nucleic acid extractor (Libex with catalog number: LOT22091210T324H).
- DNA quality and concentration was checked and maintained using the Nandrops or Qubit Machines.

- qPCR was used for genotyping and detecting SLC6A4 (5HTTLPR VNTR, rs6354; rs8076005) alleles and genotypes.
- Serum/Plasma Serotonin and Interleukin (IL-6) levels were quantified using Enzyme-Linked Immunosorbent Assay (ELISA kits).
- Serum Testosterone, Estrogen and Progesterone concentrations were estimated using Chemiluminescent Microparticle Immunoassay (CLIA Microparticles).

3.11 Copy Number Variation and Single Nucleotide Polymorphism (SNP) Sequencing and Selection

The CNV and both SNPs assays for genotyping were precisely done using custom orders from Thermo Fisher Scientific Inc. (see appendix 6), with forward primer: 5'GAAACCCAATTGGCAGAAACTC 3' and backward primer: 3'GAAGATCTGAGCGGCTGCAT 5'
for the CNV and selection of SNPs was also done based evidence and availability of pre-existing literature on the subject matter.

The two SNPs, rs6354 and rs8076005 were selected based on their potential functional association and significance with a myriad of psychiatric disorders. These SNPs were selected for their:

1. Functional Significance: the rs6354 is located at the promoter region, which may have effect on the gene expression of the SLC6A4 gene, while the rs8076005 is at the untranslated 3'region (UTR) and may be involved in managing the stability and translation of mRNA.

2. Association with Psychiatric Disorders: rs6354 has been repeatedly associated with depression, anxiety, anxiety-related disorders and Obsessive-Compulsive Disorders

(OCD). rs8076005 has also had links with depression and Attention Deficit Hyperactivity Disorder (ADHD) and Autism Spectrum Disorder (ASD).

3. Linkage Disequilibrium: Both the rs6354 and rs8076005 are not in strong linkage equilibrium with one and other, this allowed for an independent analysis of their respective effects on the study population [8, 9].

3.12 Managing and Storing Samples

Foam was avoided on or inside the specimens. Prior to start of the experiment, samples were held at room temperature (15–25°C). After nucleic acid extraction, were used right away (subjected to quality control testing and then prepped for qPCR) or refrigerated at 2-8°C for a subsequent experiment within 24 hours. The samples kept were held at -20°C for extended storage.

Materials

Pipette ranges: 20µl, 100 µl, 200 µl or 1000 µl, tips ranges: 20µl, 100 µl, 200 µl or 1000 µl, vortexer , sample holder, 75% ethanol, non-powder gloves, protective covering.

3.13 Whole Blood Extraction

The TIALONG automated nucleic acid extractor (Libex) was used in conjunction with the whole blood genomic DNA extraction kit. The extractor's interior was cleaned with 75% ethanol following the experiment and then exposed to UV light for fifteen minutes. A single run of an automatic nucleic acid extractor may process ninety-six (96) samples, automating the entire purification process, therefore, two runs were carried out to accommodate all 164 samples.

Genomic DNA was extracted using the kit. Use single-use utensils and a sample injector, as well as autoclave-processed centrifuge tubes and tips, to prevent RNase from degrading RNA during the procedure. A protective cover, a mask, and gloves free of powder were used throughout the handling of samples and reagents avoid contamination.

The kit contained a special buffer solution and magnetic beads with a separation function to extract, isolate, and purify high-quality nucleic acids from whole blood samples. High-quality nucleic acids that are devoid of protein, nuclease, and other contaminants were purified using magnetic beads. Numerous standard procedures, including as enzyme extraction, polymerase chain reaction (PCR), DNA library creation, southern hybridization, and blotting, can benefit greatly from the usage of purified nucleic acid[9].

Principles of Testing

The TIALONG automated nucleic acid extractors (Libex GeneRotex 96) and other similar instruments made by Xi'an Tialong science and technology co, ltd) were used with the whole blood genomic DNA extraction kit. Based on the principle of magnetic bead adsorption, magnetic beads are adsorbed, transported, and released by unique magnetic rods during the nucleic acid extraction process. Through the transfer of magnetic beads

and nucleic acids, the extraction process facilitates the conduction of nucleic acid extraction and the ultimate adsorption of highly pure nucleic acids.

Preparing the Reagent

96deep well plate: To re-suspend the magnetic beads, the kits were carefully inverted several times after removing the pre-filled reagent from the plastic container. A 96-well plate horizontal centrifuge was also used for centrifugation at 500 rpm for one minute. Then gently shaken to concentrate the reagent and magnetic beads on the bottom of the plate. To prevent liquid splashing, the aluminium foil sealing sheet was carefully ripped off before using.

Filling the reagent 96-deep well plate with samples:

To columns 1 and 7 of the pre-filled reagent, 15 µl of proteinase K solution was added, alongside 60 µl of nucleic acid releaser, and 200 µl of the room-temperature-equilibrated sample.

Protocol: Whole Blood Extraction using Tialong Libex

Reagents: Proteinase K solution (provided with kit), Nucleic Acid Releaser (provided with kit), Lysis buffer (provided with kit), Binding buffer (provided with kit), Washing buffer (provided with kit), Elution buffer (provided with kit)

Instrument Parameters: Protocol was used "Whole Blood", Extraction Volumewas 2 mL, Elution Volume was 100-200 µL, Lysis Time was 10-15 minutes, Binding Time at 5-10 minutes, Washing Time was set to 5-10 minutes, Elution Time also 5-10 minutes

Step-by-Step Protocol:

Extraction Protocol:

Step 1: Sample Loading: The following were added into the nucleic acid extraction rack: 2 mL of whole blood, 20 µL of Proteinase K solution, 200 µL of Nucleic Acid Releaser. The above listed components (a-c) were thorougly mixed by inverting the tube 5-10 times.

Step 2: Instrument Setup: "Whole Blood" protocol on the Libex instrument was selected, Extraction volume was set to 2 mL with an Elution volume of 200 µL.

Step 3: Extraction: The sample rack was placed in the sample compartment, the extraction machines combs were unwrapped and slotted into holders, the extraction was run according to the instrument's instructions. The nucleic acid extractor performed the following steps: Lysis (10-15 minutes); Binding (5-10 minutes); Washing (5-10 minutes) and Elution (5-10 minutes).

Step 4: Elution and Collection: The extracted nucleic acid was eluted into a collection tube. The eluated 200 µL was collected.

Post-Extraction Steps:

The extracted nucleic acid was stored at -20°C or kept on ice to prevent denaturation in between and during preparations for other protocols the later was used when waiting time was 5 minutes.

Table 3.1. Kit contents for Whole Blood Extraction

Short code Name of Component		T146H	T147H	T148H	T149H	
Pre-filled reagent	**Size**	64/Box(pre-filled)	20T/Box(pre-filled)	40T/B0x(pre-filled)	20T/Box(pre-filled)	
	Component	Pre-filled 96-deep well plate	Pre-filled 96-deep well plate	Pre-filled 96-deep well plate	Pre-filled 6 strip tube	
	Quantity	4	4	4	20	
	Component specification	16 Tests	5 Tests	10 Tests	1Test	
Proteinase k solution	**Component specification**	0.96mL	0.3mL	0.6mL	0.3mL	
	Quantity	1	1	1	1	
Nucleic Acid Releaser	**Component specification**	1.28mL	1.2mL	1.2mL	1.2mL	
	Quantity	3	1	2	1	
Corrugated paper		1 piece	1 piece	1 piece	1 piece	
White Board		1 piece	1 piece	1 piece	1 piece	
Packaging Box		1	1	1	1	**Sour ce**[9]
Instructions for use		1 copy	1 copy	1 copy	1 copy	

Table 3.2. The Extraction Procedure of Libex Nucleic Acid Extractors:

No	Column	Name	Waiting (s)	Mixing (s)	Magnet (s)	Speed	Volume (µl)	Heating state	Temp (℃)
1	2	Remove bead	0	60	90	8	600	Closed	0
2	1	Lysis	0	1200	90	7	750	Lysis	75
3	3	Washing 1	0	180	90	7	600	Closed	0
4	4	Washing 2	0	120	90	7	600	Elution	75
5	5	Washing 3	0	0	30	7	600	Elution	75
6	6	Elution	0	300	300	7	100	Elution	75
7	2	Release bead	0	60	0	7	600	closed	0

Source[9]

3.14 DNA Quality and Concentration

DNA integrity was checked and maintained using the Nandrop. Following DNA extraction and elution of DNA. Eluate is transferred into a micro-centrifuge tube[10].

NanoDrop One Settings

Instrument Name: NanoDrop One UV-Vis Spectrophotometer.

Measurement mode: DNA.

Wavelength: 260 nm, 280 nm, and 230 nm.

Measurement type: Absorbance.

Measurement Protocol

NanoDrop One was calibrated and validated using molecular graded water, 1 µL of eluted. DNA was placed onto the NanoDrop pedestal, The pedestal was closed. OD at 260/280 and OD 260/230 ratios, concentration, and purity was recorded.

Interpreting of Results

DNA Quality Indicator: Status

1. OD 260/280 Ratio: 1.8-2.0 (ideal)
   - < 1.8: Protein contamination
   - > 2.0: RNA contamination
2. OD 260/230 Ratio: 2.0-2.2 (ideal)
   - < 2.0: Salt contamination
3. Concentration: sufficient for downstream applications (e.g., PCR, sequencing)
4. Purity: % (based on OD 260/280 and OD 260/230 ratios)

Expected Results (Tialong Libex)

1. OD 260/280 ratio: 1.85-1.95

2. OD 260/230 ratio: 2.05-2.15

3. Concentration: 50-200 ng/µL (dependent on sample type and extraction efficiency)

4. Purity: ≥ 90%

Troubleshooting

1. Low OD 260/280 ratio: Protein contamination (optimize proteinase K digestion)

2. High OD 260/280 ratio: RNA contamination (optimize RNAse digestion)

3. Low concentration: Inadequate DNA extraction or excessive dilution.

3.15 qPCR Procedure for SLC6A4 (CNV)

qPCR was done using TaqMan Probes Copy Number Variation (CNV) and Single Nucleotide Polymorphism (SNP) Analysis on QuantStudio 7[10, 11, 12].

CNV Analysis

1. Instrument: QuantStudio 7 (Applied Biosystems)

2. Run MODE: Fast 96-well plate

3. Analysis Mode: Absolute Quantification (CVN)

4. Reaction Volume: 20 µL

SNP Analysis

Instrument: QuantStudio 7(Applied Biosystems)

Run MODE: Fast 96-well plate

Analysis Mode: Allelic Discrimination (SNP)

Reaction Volume: 20 µL.

Materials: DNA samples (genomic DNA or purified DNA), TaqMan CNV Assays (probe and primer sets), TaqMan Genotyping Master Mix and Optical 96-well plates and seals.

Procedures

Procedures for Entering of DNA Templates/Recommendation

The quantity of sample DNA in each well employs the same assay must be comparable, a final DNA concentration for each reaction in genotyping assays of 0.4 ng/µL was used, 1 ng/µL of final purified DNA was used for each reaction in copy number studies, Four duplicates of each reaction were done to validate for copy number experiments. Reactions were prepared in accordance with the needs of the experiment. The component sizes were adjusted based on the quantity of reactions and account for a 10% excess. A 10% overage was included and all components were scaled proportionately for reaction volumes that differ from those specified. Freeze-Thaw cycles of the master mix were minimized as excessive cycles may affect the fluorescence probes. Two no template controls (NTCs) were present as well as DNase-free water. The dilution of 40x or 80x Predesigned CNV/SNP Genotyping Assay to a working stock of 20x solution. Except for the DNA sample, NTC reactions include all reaction ingredients (master mix, assays, and water).

The total volume of each component was calculated, as needed for each assay, using (**Table 3.3**).

Note: Excess volume was prepared to account for pipetting errors.

Protocol

Step 1: Preparation: DNA samples were diluted to 10 ng/µL. TaqMan CNV Assay mix was prepeared according to manufacturer's instructions.

Assembly of the PCR Reactions.

Compile the necessary number of reactions in accordance with the relevant table, plus an additional 10%. After proper combination of ingredients, the mixture was centrifuged to remove any air bubbles. Each optical plate well was filed with the appropriate volume of each reaction. An optical adhesive cover was used to seal the plate, and then quickly centrifuged to get rid the contents of any air bubbles. The reaction plate was kept at room temperature for up to 72 hours.

Table 3.3. PCR Reactions for Copy Number Experiments

Component	Volume per reaction	
	10 µl	**20.0 µl**
TaqPath–ProAmp-Master Mix	5.0 µl	10.0 µl

TaqMan-copy number Assay(1) (20X)	0.5 µl	1.0 µl
TaqMan-copy number Reference Assay(1) (20X)	0.5 µl	1.0 µl
Genomic DNA-or-NTC	Up 4 µl	Up to 8 µl
Nuclease-free water	to 10 µl total	To 20 µl total
Total volume	10 µl	20 µl

Source[12]

Table 3.4. PCR Reaction for Genotypying Experiments

Component	**Volume per reaction**		
	5µl	**10 µl**	**25 µl**
TaqPath–ProAmp-Master Mix	2.5 µl	5.0 µl	12.5 µl
TaqMan-SNP-Genotyping Assay(1) (20X)	0.25 µl	0.5 µl	1.25 µl

Genomic DNA-or-NTC	Up to 2.25 µl	Up to 4.5 µl	Up to 11.25 µl
Nuclease-free water	to 5 µl total	to 10 µl total	to 25 µl total
Total volume	**5 µl**	**10 µl**	**25 µl**

Source[12]

Configuration and Operation of the Real-Time PCR Device.

The reaction plate was placed in real-time PCR machine. The proper PCR thermal cycling parameters were establish. It should be noted that heat-labile UNG was fully inactivated during the first ramp leading up to the 95°C hold step, and was active during the reaction setup.

Table 3.5. Genotyping Experiments: Fast cycling

Step	Temperature	Time	Cycles
Pre-Read	60°C	30 seconds	hold
Initial denature/Enzyme activation	95°C	5 minutes	
Denature	95°C	5 seconds	40
Anneal/Extend	60°C	30 seconds	
Post-Read	60°C	30 second	Hold

Source[11,12]

The volume of the reaction was adjusted to suit the reaction plate and then ran.

Conduction of Allelic Discrimination.

Allelic discrimination was carried out in genotyping experiments with a post-read temperature of 60°C.

Examining the Results.

Analysing of data was done on the Data and Analysis Software 2.8.0

3.16 Serum/Plasma Serotonin and Interleukin (IL-6) levels using Enzyme-Linked Immunosorbent Assay (ELISA kits)

The assays and protocols used for serum/plasma serotonin and interleukin 6 were provided by SunLong BioTech.CO., LTD and all instructions were carefully complied with and are as follows:

Table 3.6. Materials Provided with the ELISA Kit

	Materials provided with the kit	96 Test Determination	Storage
1	User manual	1	R.T.
2	Closure plate membrane	2	R.T.
3	Sealed bag	1	R.T.
4	Microelisa stripplate	1	2-8℃
5	Standard:90ng/L	0.5ml×1 bottle	2-8℃
6	Standard diluent	1.5ml×1 bottle	2-8℃
7	HRP-Conjugate reagent	6ml×1 bottle	2-8℃
8	Sample diluent	6ml×1 bottle	2-8℃
9	Chromogen solution A	6ml×1 bottle	2-8℃
10	Chromogen solution B	6ml×1 bottle	2-8℃
11	Stop solution	6ml×1 bottle	2-8℃
12	Wash solution	20ml (30X) ×1 bottle	2-8℃

Source[13, 14]

Sample Preparation

1. Preparing Serum

Once the whole blood was allowed to clot by keeping it undisturbed at room temperature, which took around 10 to 20 minutes. Centrifuging the clot for 20 minutes at 2,000–3,000 rpm removed it. In situations, where the precipitate formed during the reservation process, the sample was centrifuged once more.

Procedure

Dilution of the Standard : First, dilution was done to standard using tiny tubes. Next, a volume of 50µl was pipetted from each tube into a microplate well, using two wells for each tube, for a total of ten wells.(**see table 3.7**).

As a blank control, a well in the microelisa stripplate was left empty. 10µl of sample (dilution factor: 5) and 40µl of samples dilution buffer are added to sample wells. Samples were placed such they don't come into contact with the well wall. Shook gently to mix well. Incubation: after the closure plate membrane had been sealed, incubation for 30 minutes at 37°C was done. Dilution: distilled water was used to dilute the concentrated washing buffer (30 times for 96T).

Cleaning: the membrane of the closure plate was gently removed, aspirated, and replenished with the cleaning solution. After the wash solution had rested for 30 seconds, it was discarded. The washing process was done 5 times. All wells were filled with 50µl

of HRP-Conjugate reagent, excluding the blank control well. Incubation was repeated as in the aformention step.

Table 3.7. ELISA Dilution Standard

60ng/L	STD No.1	300µl Original STD+150µl STD diluent
40ng/L	STD No.2	300µl STD No.1+ 150µl STD diluent
20ng/L	STD No.3	300µl STD No.2+ 150µl STD diluent
10ng/L	STD No.4	300µl STD No.3+ 150µl STD diluent
5ng/L	STD No.5	300µl STD No.4+ 150µl STD diluent

Source[13,14]

Washing as repeated (30 minutes for 96T). Colour, was done by filling each well with 50 µl of each chromogen solution A and B. Shook gently to mix, then incubated for 15 minutes at 37 °C. Light was avoided while colouring took place. Stop: To halt the reaction, 50µl of stop solution was introduced into each well. The well's colour was expected to shift from blue to yellow. Utilising a Microtiter plate reader, the absorbance was determined, O.D. at 450 nm. The blank control well's OD value is set to zero.

Precautions

Upon receiving the kit, it was stored at 4°C. Before the assay, the kit was brought to room temperature. Extra strips were taken off from the plate coated with the human serotonin and IL-6 antibody. Concentrated washing buffer that formed precipitates. To ensure that all of the precipitates were dissolved, the buffer was heated. To prevent experimental error, accurate pipettes were used. In less than five minutes, samples were introduced to the microplate. Every assay needed to include a standard curve, replicate wells were used. Microplate sealers were only once to prevent cross-contamination. The substrate was protected from sunlight.

Calculations of the Results

The log scale (X-axis) and log scale (y-axis) are used to plot the known concentrations of human IL-6 and serotonin standard and the corresponding reading, respectively. The O.D. of the sample is plotted on the Y-axis to determine the concentration of human IL-6 and serotonin in the sample. Determination of the initial concentration by multiplying the dilution factor.

Accuracy

Three samples with low, intermediate, and high levels of human IL-6 and serotonin were evaluated 20 times each on a single plate, demonstrating intra-assay precision (precise inside an assay).

Inter-assay precision, or the precision between assays: three samples three with human IL-6 and serotonin levels that were low, middle, and high were examined on three separate plates, each with eight duplicates.

CV (%) = mean X 100 - SD

Intra-assay: CV<10%

Inter-assay: CV<12%

Test range:

2 ng/L to 80 ng/L

Sensitivity

0.6 ng/L

3.17 Serum Testosterone, Estrogen and Progesterone using Chemiluminescent Microparticle Immunoassay (CLIA Microparticles)

Progesterone (PRG)/Tetestorone/Estrogen (Estradiol/E2) CLIA Microparticles Immunoassay protocol was provided by AutoBio Diagnostics CO., LTD and all instructions were carefully complied with and are as follows:

The one-step competitive approach served as the foundation for the test. The mixture consisted of the sample, microparticles coated with mouse anti-sheep antibodies, antibody solution, and enzyme-labelled PRG (in the case of progesterone); sample, goat polyclonal anti-mouse IgB antibodies coated Microparticles, Antibody Solution and enzyme labelled testosterone (in the case of testosterone); the sample mouse ant-rabbit antibodies coated Microparticles, antibody solution and enzyme labelled E2[15,16,17].

Materials

1. Calibrators

Seven (7) vials with 1.0 ml of calibrators A through G in each. Human serum devoid of hormones is mixed with PBS (phosphate buffered saline) buffer to form the matrix, included was a variety of preservatives.

3.8. Reagent pack for CLIA micro particles.

Reagent pack provided ready to use

	50*1	100*1	100*2	100*5	50*2
Microparticles	1.2ml*1	2.3ml*1	2.3ml*2	2.3ml*5	1.2ml*2

Solution					
Enzyme Conjugate	3.0ml*1	5.5ml*1	5.5ml*2	5.5ml*5	3.0ml*2
Antibody Solution	3. 0m1*1	5.5ml*1	5.5ml*2	5.5ml*5	3.0ml*2

Source[15,16,17]

Micro particle solution

Microparticles coated with goat polyclonal anti-mouse IgG antibodies (Testosterone)/mouse anti-rabbit antibodies (E2) in PBS buffer containing BSA and mouse anti-sheep antibodies (PRG). includes a variety of preservatives.

Enzyme conjugate

Preservatives are included in the horseradish-peroxidase-labeled PRG/E2/Testosterone in MES buffer with BSA.

Antibody solution

Monoclonal antibodies against goat, mouse, and rabbit in tris-NaCL buffer with BSA. ProClin 300 and Bronidox preservatives are present.

Other Materials Required

Analyzer for Assays, The reaction vessel(s) used to combine the sample and reagent, Sample tubes or cups for the sample that include :Chemiluminscent substrate, System wash for cleaning the pipette, The wash buffer that was utilised to wash the process and Deionized water.

Sample

Serum samples were gathered in compliance with accepted medical procedure. Samples with evident microbial contamination were not used. Samples containing sediments and suspended solids that could affect the test were centrifuged off. Before centrifuging, the serum samples have fully formed clots. Turbid, lipemic, or severely hemolytic materials were not used.

After capping the samples, they were kept at 18–25°C for no more than 8 hours. If the samples were to be used for a longer period of time, then they were stored at 2–8°C for up to 48 hours, Freeze-thaw samples were properly mixed using a low-speed vortex or ten inversions. To guarantee uniformity in the results, the thawed samples were centrifuged that included red blood cells, particle matter, or that seemed hazy or murky before using them.

Procedure for measurements

1. Load the kits

Before placing the new (punctured) reagent parks onto the analyzer, the contents were mixed by gently inverting the park multiple times. Production of foam in all reagents was prevented. Scanning was done automatically for the code on the reagent bag to retrieve the necessary test settings. In rare circumstances where the code could not be read, they were manually recognised.

2. Place tests in order.

50 µl of each test' samples were placed in sample cups or tubes on the sample rack. Sample information was filled onto the system software interface after loading the sample rack.

"Run" was chosen to initiate the test; tests are conducted automatically by the analyzer. It carried out the subsequent tasks:

4. Adjust the curvature were done according to the manual.

5. Dilute the sample

Samples that have a PRG and E2 value more than 120ng/ml and 4500 pg/mL respectively manually diluted. Samples can be diluted with low-value or hormone-free serum. The outcome should be multiplied by the dilution factor after dilution. After dilution, the sample's concentration were not less than 2ng/ml for (PRG).

Measurement of outcomes

The system software determined the sample test results automatically.

Endnotes

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Chapter Four

Results and Discussion of Findings

4.1 Results of Findings

This chapter presents the findings of the study examining the role of the SLC6A4 gene, serotonin levels, gonadocorticoids, and inflammation in the severity of depression among patients from Nigeria. The results of this comprehensive analysis provide novel insights into the complex interplay between genetic, biochemical, and demographic factors that contribute to the occurrence and severity of depression. The following sections will detail the key findings, highlighting significant associations, correlations, and trends that emerged from the data.

Reasons for Sample Discrepancy

Participant Withdrawal or Attrition

Eight (8) of the study's participants were excluded post-recruitment due to consent withdrawal, contamination, or clinical disqualification. This brought the origianlly calculated sample size of 172 to 164. This was well accomodated by the attrtion.

Missing or Incomplete Data

Some participants may have had incomplete questionnaire responses, missing demographic details, such answers were recorded as unknown especially in the sections that dealt with alchohol and smoking status.

Failed Laboratory Assays / Sample Amplification

Some DNA samples did not amplify during genotyping (6 failed amplifications for rs8076005, 4 for rs6354). This is a technical limitation common in PCR-based analyses and as such caused a distortion to the total for SNP figures.

Serum Quantity Constraints

In some cases, insufficient blood or serum volume was available for biochemical assays (e.g., IL-6, serotonin, hormone levels).

Sex- or Age-Stratified Analyses

Subgroup tables (e.g., male-only ANOVA or female-specific hormone analyses) naturally exclude participants of the opposite sex or non-stratifiable ages, reducing the effective sample size in those contexts.

Data Quality Control or Outlier Removal

Some values may have been removed due to outlier exclusion, statistical inconsistencies, or quality control procedures, inorder to avoid skewing of results.

4.1: Study Populations' Enrolment Data

The frequency table (**Table 4.1**) showed the distribution and characteristics of the study population with 73 (44.5%) enrolled as cases and 91 (55.5%) enrolled as the control.

The Hamilton Depression Rating Scale (HDRS-17) which categorises depression (normal/clinical remission: 0-7; moderate: 8-19; severity: 20 and above)**,** grouped the study participants into 60 (36.6%), 55(33.5%) and 49(29.9%) for normal, moderate and severe respectively[1].

The study population consisted of 37(22.6%) males and 127(77.4%) females, Age was further stratified, Men were grouped into 40 and above and 40 and below while women were grouped into 20 and above and 20 and below. This was to give account to possible sex differences to flagship disparities in hormonal levels. 68(41.4%) had children and 96(58.6%) said they did not (**Table 4.1**).

Marital status in this study which included being; single, married and divorced/widowed groups divided the population into 97(59.1%), 58(35.4) and 9(5.5) respectively.

Smoking and alcohol consumption were taken into consideration as risk factors to depression. 60 (37.5%) drank alcohol, 100 explained they did not, while 4 out of the 164 participants were unaccounted for. Only 18 (11.2%) of the study participants agreed to have been smokers, although the degree of, how often they smoked was not inquired, while 142 (88.8%) had affirmed they did not smoke.

Table 4.1: Study Populations' Enrolment Data

Variables	n	%
Group		
Cases	73	44.5
Control	91	55.5
HDRS		
Normal	60	36.6
Moderate	55	33.5
Severe	49	29.9
Sex		
Male	37	22.6
Female	127	77.4
Age band		
Male < 40 yrs.	19	51.4
Male ≥ 40 yrs.	18	48.6
Female: < 20 yrs.	39	30.7
Female: ≥ 20 yrs.	88	69.3
Marital status		
Single	97	59.1
Married	58	35.4
Divorced/widowed	9	5.5
Children		
With Children	68	41.4
Without Children	96	58.6
Employment status		
Not employed	64	39.5
Employed	98	60.5
Alcohol consumption		
Yes	60	37.5
No	100	62.5

Smoking status		
Yes	18	11.2
No	142	88.8

n: frequency number ; %: percentage; HDRS: Hamilton Depression Rating Scale.

Source: Author's Laboratory Work, 2024.

Figure 4.1: Average Age Among the Study Population

In (**figure 4.1)**, the average age of the study population, for women who were diagnosed with depressed was around 30 years old, the control group had a mean age of 26.7 years. On the other hand, the men who were diagnosed with depression were 35.7 years old and those enrolled in the control group had an average were 43 years.

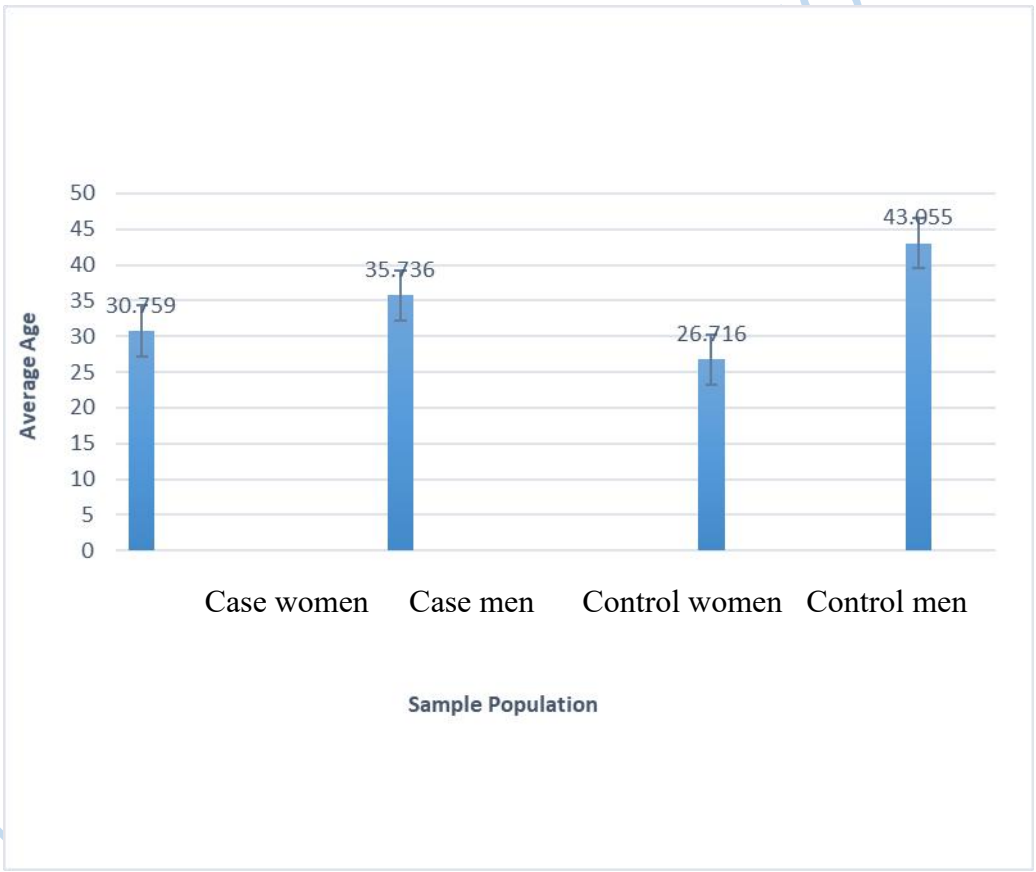


Figure 4.1: Average Age Among the Study Population

Source: Author's Laboratory Work, 2024.

Figure 4.2: Hamilton Depression Rating Scale (HDRS) Among Test & Control Group

The frequency of the Hamilton Depression Rating Scale among the test and control group, (**figure 4.2)**, shows the distribution of the normal, moderate and severe scale to be 55,18 and 0; 0,19 and 36, 4,12 and 2; and 2,5 and 12 for the women enrolled as control; women diagnosed with depression; men enrolled as control and men diagnosed with MDD.

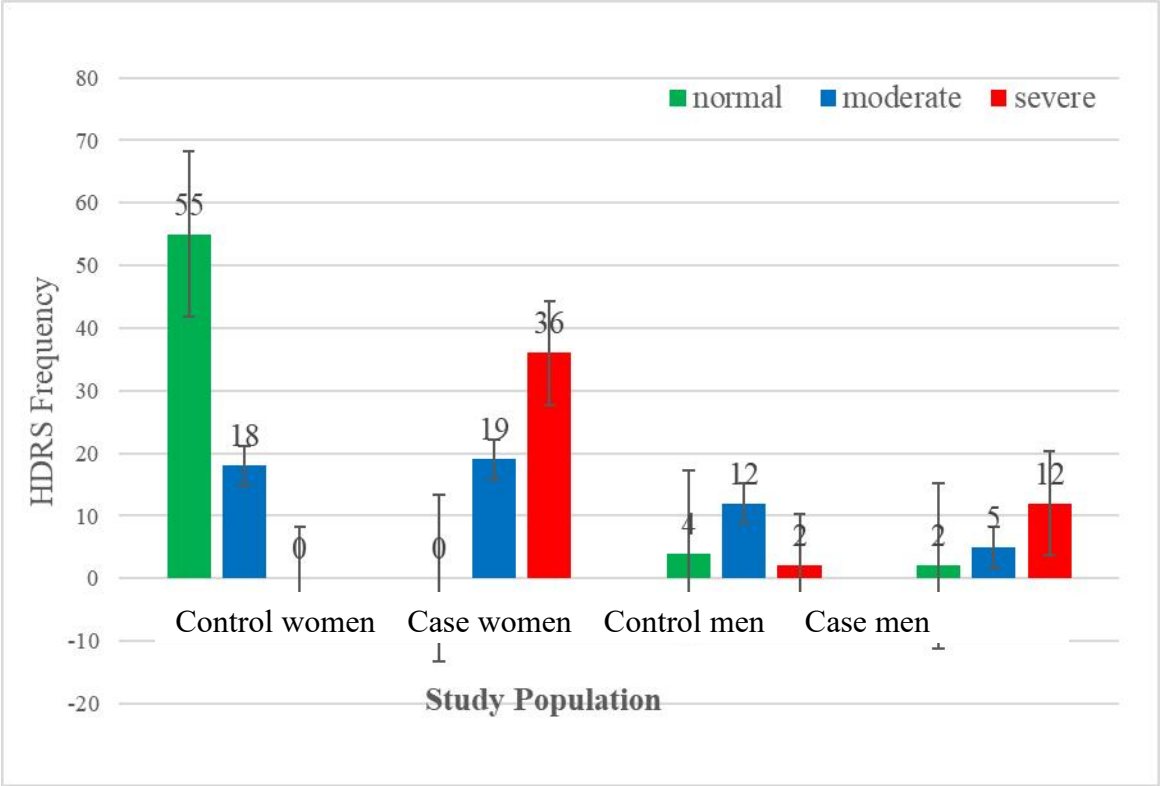


Figure 4.2: Hamilton Depression Rating Scale (HDRS) Among Test & Control Group

Source: Author's Laboratory Work, 2024

4.2: Study Populations' SLC6A4 Genomic Data

Genotyping was carried out on all 164 of the study's cohorts for the SLC6A4 gene (**Table 4.2**). The 5HTTLPR indel region and 2 SNPs (rs8076005 and rs6354) were of interest. The indel region showed zero copy numbers and was found to not be present in this study population, which could inform a novel finding, especially as this is the is the first record of genotyping at this locus in Nigeria. Neither the long or short allele were observed in the cohorts.

The rs8076005 has 2 alleles A and G, and found three genotypes in the population. Homozygous AA and GG and heterozygous AG. (**Table 4.2)**, showed that the study population had 10(6.21%) individuals with AA phenotype; 49 (30.43%) with AG phenotype; and 96(59.63%) with the GG phenotype, however 6 out of the 164 were unable to amplify.

The rs6354 also had 2 alleles G and T, three phenotypes were found in the population. Homozygous GG and TT phenotypes and heterozygous GT phenotype. In this populace, 22(14.10%) individuals were genotyped to have the GG phenotype; 45(28.85%) to have GT and 85(54.49%) to have phenotype TT with the rest not amplifying.

Table 4.2: Study Populations' SLC6A4 Genomic Data

Variables	n	%
5HTTLPR-VNTR	0	100
Long Allele (528 bp)	0	0
Short Allele (484 bp)	0	0
rs8076005		
AA	10	6.21
AG	49	30.43
GG	96	59.63
NA	6	3.73
rs6354		
GG	22	14.10
GT	45	28.85
TT	85	54.49
NA	4	2.56

n: frequency number ; %: percentage; NA: Not Amplified.

Source: Author's Laboratory Work, 2024.

4.3: SNP Phenotype Distribution between the Case and Control

The phenotypic distribution (**Table 4.3**), of rs8076005 were 4, 21 and 47 in case and 13,19 and 35 in control for AA, AG and GG phenotypes. The phenotypic distribution of

rs6354 were 13, 19 and 35 in case and 9, 26 and 50 in control for GG, GT and TT phenotypes respectively.

Table 4.3: SNP Phenotype Distribution between the Case and Control

Variables	Case n(%)	Control n(%)	x^2	*P*
rs8076006				
AA	4(40)	6(60)	0.66	0.7174
AG	21(42.86)	28(57.14)		
GG	47(48.96)	49(51.04)		

rs6354				
GG	13(59.09)	9(40.91)	2.36	0.307
GT	19(42.22)	26(57.78)		
TT	35(41.18)	50(56.82)		

n:frequency number; %: percentage; x^2: chi square test; p: p-value.

*P <0.05
**P <0.01
****P* <0.001

Source: Author's Laboratory Work, 2024.

4.4: SNP Phenotype Distribution between the HDRS Groups

The SNP phenotype distribution (**Table 4.4**), provided an avenue to observe possible associations between the study participants grouped based on their HDRS and their genotypes, however, rs8076005 (p = 0.113), and rs6354 (p = 0.479) both showed no significant associations.

Table 4.4: SNP Phenotype Distribution between the HDRS Groups

Variables	Normal n(%)	Moderate n(%)	Severe n(%)	x^2	*P*
rs8076006					
AA	6(60.0)	2(20.0)	2(20.0)	7.47	0.113
AG	21(42.86)	14(28.57)	14(28.57)		
GG	25(29.07)	36(37.50)	35(36.46)		
rs6354					
GG	6(27.27)	6(27.27)	10(45.45)	3.39	0.479
GT	15(33.33)	19(42.22)	11(24.44)		
TT	30(35.29)	29(34.11)	26(30.59)		

n: frequency number; %: percentage; x^2: chi square test; p: p-value.

*P <0.05
**P <0.01
****P* <0.001

Source: Author's Laboratory Work, 2024.

4.5: rs8076005 Hardy Weinberg Phenotype and Allele Distribution between the Depression Strata and Control

Null hypothesis for the rs8076005's Hardy Weinberg Phenotype and Allele Distribution (**Table 4.5**), between the Depression Strata (moderate and severe depression) and Control was completely accepted (p = 0.874; 0.839 and 0.783).

Moderate depression population had 2(2.6%) individuals with AA phenotype, 8(34.8%) individuals with AG phenotype, 13(56.5%) individuals with GG, with allele frequency A and G being 0.26 and 0.74 correspondingly.

Severe depression population had 2(4.0%) individuals with AA phenotype, 13(26.5%) individuals with AG phenotype, 34(69.4%) individuals with GG, with allele frequency A and G being 0.17 and 0.83 sequentially. Control populations had 6(7.2%) individuals with AA phenotype, 29(34.9%) individuals with AG phenotype, 48(57.8%) individuals with GG, with allele frequency A and G being 0.24 and 0.76 correspondingly.

Table 4.5: rs8076005 Hardy Weinberg Phenotype and Allele Distribution between the Depression Strata and Control

Group	Genotypes distribution			Allele frequency			
	AA n(%)	AG n(%)	GG n(%)	A	G	x^2	P
Moderate Depression	2 (2.6)	8(34.8)	13(56.5)	0.26	0.74	0.27	0.874
Severe Depression	2(4.0)	13(26.5)	34(69.4)	0.17	0.83	0.35	0.839
Control	6 (7.2)	29(34.9)	48(57.8)	0.24	0.76	0.45	0.783

n: frequency number; %: percentage; x^2: chi square test; p: p-value.

*P <0.05
**P <0.01
***P <0.001

Source: Author's Laboratory Work, 2024.

4.6: rs6354 Hardy Weinberg Phenotype and Allele Distribution between the Depression Strata and Control

Alternate hypothesis for the rs6354's Hardy Weinberg Phenotype and Allele Distribution (**Table 4.6**), between the Depression Strata (moderate and severe depression) and Control was partly accepted (p = 0.758, 0.035* and 0.145).

Moderate depression population had 3(13.6%) individuals with GG phenotype, 8(36.6%) individuals with GT phenotype, 11(50%) individuals with TT, with allele frequency G and T being 0.32 and 0.61 correspondingly. Severe depression population had 9(20%) individuals with GG phenotype, 11(24.4%) individuals with GT phenotype, 25(55.6%) individuals with TT, with allele frequency G and T being 0.32 and 0.68 sequentially.

Control populations had 9(10.5%) individuals with GG phenotype, 26(30.6%) individuals with GT phenotype, 50(58.8%) individuals with TT, with allele frequency G and T being 0.26 and 0.74 correspondingly. Here, rs6354, has a significant association with the severe depression (p=0.035).

Table 4.6: rs6354 Hardy Weinberg Phenotype and Allele Distribution between the Depression Strata and Control

Group	Genotypes distribution			Allele frequency			
	GG n(%)	GT n(%)	TT n(%)	G	T	x^2	p
Moderate Depression	3(13.6)	8(36.3)	11(50)	0.32	0.61	0.55	0.758
Severe Depression	9(20)	11(24.4)	25(55.6)	0.32	0.68	6.69	**0.035***
Control	9 (10.5)	26(30.6)	50 (58.8)	0.26	0.74	3.87	0.145

n: frequency number; %: percentage; x^2: chi square test; p: p-value.

*P <0.05
**P <0.01
****P* <0.001

Source: Author's Laboratory Work, 2024.

4.7: Distribution of Severity of Depression by Cohort and Sex

Severity of depression was statistically significant (p = <0.001**) in the case group than to the control (**Table 4.7**) alongside the sex groups (p = 0.013*).

Table 4.7: Distribution of Severity of Depression by Cohort and Sex

	HDRS					
	Normal	**Moderate**	**Severe**	**Total**		
	n (%)	n (%)	n (%)	n (%)	$\chi 2$	**P**

Control	58 (35.4)	31 (18.9)	2 (1.2)	91 (55.5)	93.64	**<0.001****
Case	2 (1.2)	24 (14.6)	47 (28.7)	73 (44.5)		
Sex						
Male	6 (3.7)	17 (10.4)	14 (8.5)	37 (22.6)	8.63	**0.013***
Female	54 (32.9)	38 (23.2)	35 (21.3)	127 (77.4)		

n: frequency number; %: percentage; x^2: chi square test; p: p-value.

*P <0.05
**P <0.01
****P* <0.001

Source: Author's Laboratory Work, 2024.

4.8: Age Distribution of Participants Stratified by Depression and Control

No association was found between sex, case and control (p = 0.354) or between male age strata (< 40 years and ≥ 40 years), case and control (p = 0413) (**Table 4.8)**.

An association was found between the female age strata (< 20 years and ≥ 20), control and case (p = <0.001**). There are 19 (51.4%) men below 40 while the rest, 18 (48.6%) are 40 and below. For women, below 20 group were 39(30.7%) and 20 and above were 88(69.3%).

Table 4.8: Age Distribution of Participants Stratified by Depression and Control

	Control	**Case**	**Total**		
Sex	**n (%)**	**n (%)**	**n (%)**	**χ2**	**P**
Male	18 (11.0)	19 (11.6)	37 (22.6)	0.91	0.354
Female	73 (44.5)	54 (32.9)	127 (77.4)		
Male					
< 40 yrs.	8 (21.6)	11 (29.7)	19 (51.4)	0.67	0.413
≥ 40 yrs.	10 (27.0)	8 (21.6)	18 (48.6)		
Female					

< 20 yrs.	37 (29.1)	2 (1.6)	39 (30.7)	32.20	**<0.001****
≥ 20 yrs.	36 (28.3)	52 (40.9)	88 (69.3)		

n: frequency number; %: percentage; x^2: chi square test; p: p-value.

*P <0.05
**P <0.01
****P* <0.001

Source: Author's Laboratory Work, 2024.

4.9: Association of Age Distribution and Presence of Children on Case and Control

In (**Table 4.9**), no association was found in the average age of the study population and case or control (p = 0.252).

No association was also found between those that had or did not have children and being a case or control.

Table 4.9: Association of Age Distribution and Presence of Children on Case and Control

Variables	Case n (Mean ± SD)	Control n (Mean ± SD)	*F*	t	*P*
Age	73 (32.05±11.70)	91 (29±12.01)	1.319	1.15	0.252
Children	68 (0.96 ±1.42)	89 (1.11 ±1.49)	0.443	-0.67	0.506

SD: Standard Deviation; F: F-statistics; t: t-test; p: p-value.

*P <0.05
**P <0.01
***P <0.001

Source: Author's Laboratory Work, 2024.

4.10: Association of Socio-Economic Factors in Case and Control Groups

Association of socio-economic factors in case and control were important to investigate and provide possible associations to understand non-biological contributing factors (**Table 4.10**). There relationship between the those who were employed and are not depressed was found (p=0.044*).

There are also significant odds that a case is almost twice as likely to being depressed (OR = 1.869; POR = 0.029*; 95%CI = 0.986-3.538). This study (**Table 4.10**) revealed were no associations between marital status or stress related issues in marital relationships (p = 0.601) and the likelihood of being depressed (OR = 1.218; POR = 0.278; 95%CI = 0.637-2.326).

(**Table 4.10**) There were no associations between being a smoker and being depressed (p = 0.162; OR = 0.456; POR = 0.078; 95CI = 0.154-1.346). In contrast, people who drink alcohol have a strong association with not being depressed (p = 0.005**). Additionally it is more likely to be a drinker and be healthy (OR = 0.272; POR = <0.001; 95%CI = 0.135-0.552) than not.

Table 4.10: Association of Socio-Economic Factors in Case and Control Groups

Variables	Case n (%)	Control n (%)	x^2	*P*	*OR*	P^{OR}	*95% CI (LL-UL)*
Employment							
Employed	37(37.76)	61(62.24)	6.23	0.044*	1.869	0.029*	0.986-3.538
Unemployed	34(53.13)	30(46.88)					
Marital Status							
Divorced/widowed	3(33.33)	6(6.66)	1.02	0.601	1.218	0.278	0.637-2.326
Married	24(41.38)	34(58.62)					
Single	46(47.42)	51(52.58)					
Smoking							
Non-smokers	65(45.77)	77(54.23)	3.64	0.1622	0.456	0.078	0.154-1.346
Smokers	5(27.78)	13(72.22)					
Alcohol Status							
Drink	15(25)	45(75)	15.21	0.005**	0.272	**<0.001****	0.135-0.552
Don't Drink	55(55)	45(45)					

n: frequency number; %: percentage; x^2: chi square test; p: p-value; OR: Odds Ratio; P^{OR}: p-value for Odds Ratio ; CL- Confidence Interval
*P <0.05
**P <0.01
***P <0.001

Source: Author's Laboratory Work, 2024.

4.11: Association of Socio-Economic Factors in HDRS Groups

In closer detail, (**Table 4.11**) relationships were explored around socio-economic variables and the severity of depression. Employment and marital status had no connections with the severity of depression ($p = 0.199$ and 0.834 respectively). Neither did smoking or alcohol consumption show to have any role in the severity of those diagnosed with MDD ($p = 0.089$ and 0.092 respectively).

Table 4.11: Association of Socio-Economic Factors in HDRS Groups

	HDRS				
	Normal n (%)	Moderate n (%)	Severe n (%)	**χ2**	**P**
Employment					
Employed	37(37.76)	34(34.69)	27(27.55)	3.23	0.199
Unemployed	16(25.00)	24(37.50)	24(37.50)		
Marital Status					
Single/Divorced/widowed	34(32.08)	39(36.79)	33(31.13)	0.36	0.834
Married	21(36.21)	19(32.76)	18(31.03)		
Smoking					
Non-smokers	10(55.56)	4(22.22)	4(22.22)	4.93	0.089
Smokers	42(29.58)	53(37.32)	47(33.10)		
Alcohol Status					
Drink	21(35.00)	26(43.33)	13(21.67)	4.76	0.092
Don't Drink	30(30.00)	32(32.00)	38(38.00)		

n: frequency number; %: percentage; x^2: chi square test; p: p-value.

*P <0.05
**P <0.01
****P* <0.001

Source: Author's Laboratory Work, 2024.

4.12: Comparison of Age and Serological Variables of Participants Across Control and Case Groups

A comprehensive association was evaluated (**Table 4.12**) between the depression and control groups against gonadocortcoids (testosterone, progesterone and estrogen) and an inflammatory hormone (interlukin-6). Serotonin levels were crucially associated with depression (p= <0.001), the average concentration of overall serotonin was decreased in the depression group (11.22 ± 1.45) as compared to the control(25.12 ± 1.58). IL-6 concentrations were showed no interconnections with MDD (p = 0.385).

General testosterone concentrations (**Table 4.12**) were decreased in and depression group presented statistical interactions (p = 0.024) as control volumes were found to be higher. Overall concentrations of progesterone and estrogen levels did not show any notable connections to depression.

In the males, age was a factor significantly associated (p = 0.032), the control group had older men (43.06 ± 7.71) with this group as compared to the the depressed (35.74 ± 11.75). The average age in the female population was significantly increased (p = 0.040) in those that were experiencing MDD (30.76 ± 11.54) than in those that were enrolled as control (26.67 ± 10.60).

Il-6 levels are equally substantially increased in the control (8.13 ± 1.51), which could be acting as a potential protecting bio-molecule against depression (**Table 4.12**).

Testosterone levels (**Table 4.12**) showed a statistical increase in men (p = <0.001**), contrary to the overall study populace, here, much higher concentrations were found in the individuals diagnosed with depression (5.89 ± 1.48) than in the control (1.74 ± 0.70).

Progesterone and oestrogen levels did not show any significance linkages in men as it relates to depression ($p = 0.407$; $p = 0.144$).

Table 4.12: Comparison of Age and Serological Variables of Participants Across Control and Case Groups

Variables	Depression Mean ± SD	Control Mean ± SD	t	*P*
n	73	91	–	–

Age (yrs.)	32.05 ± 11.70	29.91 ± 12.01	1.15	0.252
Serotonin (ng/mL)	11.22 ± 1.45	25.12 ± 1.58	12.23	**<0.001****
Interleukin–6 (ng/mL)	8.32 ± 1.55	7.94 ± 1.48	0.92	0.358
Testosterone (ng/mL)	1.78 ± 0.45	2.95 ± 0.98	2.28	**0.024***
Progesterone (ng/mL)	2.63 ± 1.13	1.86 ± 0.88	1.15	0.254
Oestrogen (ng/mL)	0.13 ± 0.20	0.12 ± 0.28	0.16	0.876
Male				
n	19	18	–	–
Age (yrs.)	35.74 ± 11.75	43.06 ± 7.71	2.23	**0.032***
Serotonin (ng/mL)	10.59 ± 1.32	25.12 ± 1.95	5.17	**<0.001****
Interleukin–6 (ng/mL)	6.61 ± 1.26	8.13 ± 1.51	2.07	**0.048***
Testosterone (ng/mL)	5.89 ± 1.48	1.74 ± 0.70	6.13	**<0.001****
Progesterone (ng/mL)	0.21 ± 0.09	1.06 ± 1.98	0.84	0.407
Oestrogen (ng/mL)	0.05 ± 0.01	0.79 ± 1.71	1.49	0.144
Female				
n	54	73	–	–
Age (yrs.)	30.76 ± 11.54	26.67 ± 10.60	2.07	**0.040***
Serotonin (ng/mL)	10.72 ± 1.48	25.12 ± 1.48	11.16	**<0.001****
Interleukin–6 (ng/mL)	8.51 ± 1.58	7.24 ± 1.45	2.10	**0.038***
Testosterone (ng/mL)	0.29 ± 0.18	0.96 ± 2.19	0.94	0.351
Progesterone (ng/mL)	2.09 ± 0.79	1.58 ± 0.91	0.74	0.462
Oestrogen (ng/mL)	0.12 ± 0.11	0.27 ± 0.73	0.89	0.374

t: t-test; SD: Standard Deviation; p: p-value.

*P <0.05
**P <0.01
***P <0.001

Source: Author's Laboratory Work, 2024.

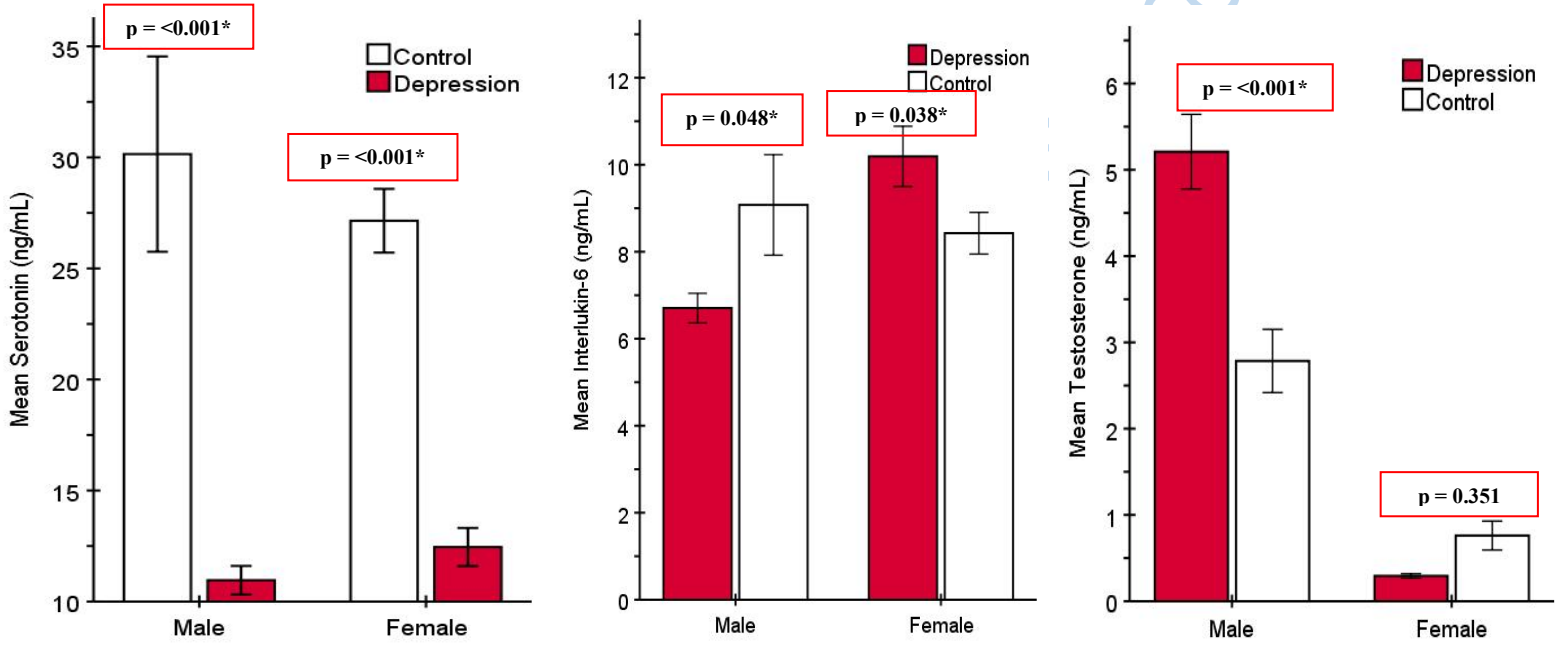


Figure 4.3: Serological Concentrations Between Depression & Control in Men and Women

Source: Author's Laboratory Work, 2024.

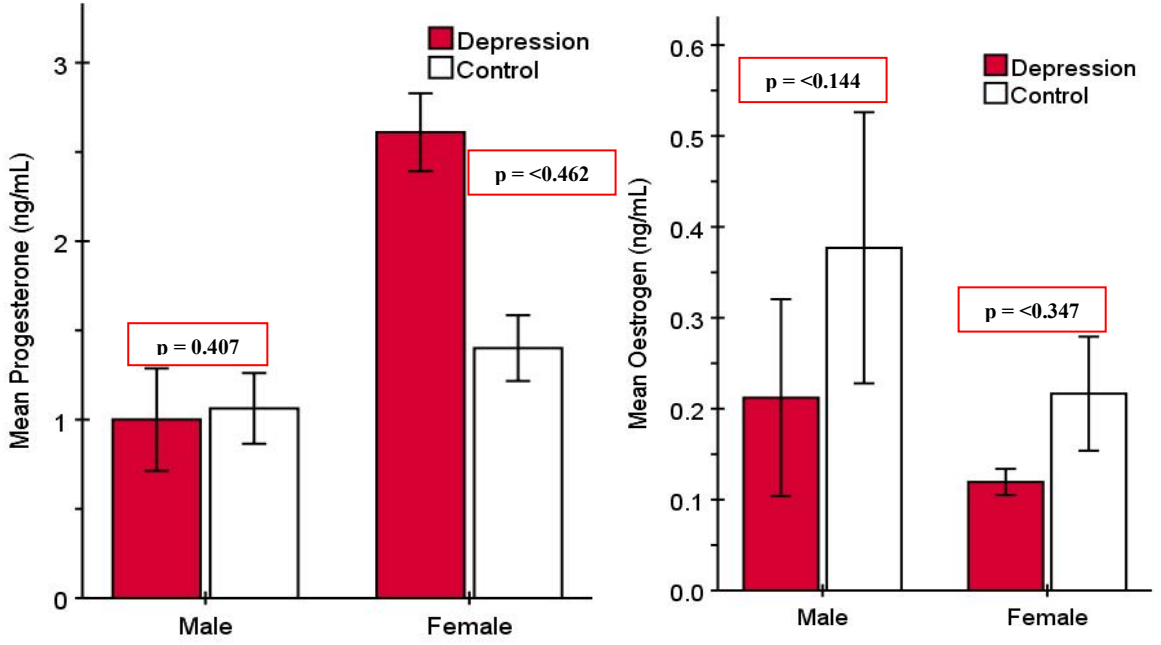


Figure 4.4: Serological Concentration Between Depression & Control in Men and Women

Source: Author's Laboratory Work, 2024.

4.13: Comparison of Variables of Participants Based on Severity of Depression for the Overall Population and Stratified by Sex

The comprehensive concentration of immunological molecules in the blood and its in the anticipation of MDD severity for this study was also taken account of (**table 4.13**). The severe group (33.53 ± 12.26) had on average a higher age range than the moderate (31.67 ± 12.33) or normal (27.95 ± 10.67) participants and was significantly associated (p = 0.041*). Serum serotonin concentrations were investigated to be significantly increased (p = <0.001**) in normal participants (23.99 ± 1.51) while reduced on the opposite spectrum (severe MDD 11.22 ± 1.45) in this study.

A more intrinsic evaluation of the the acuteness of depression as regards sex differences was also taken into account (**Table 4.13**) Average serum serotonin (25.12 ± 2.24) and oestrogen (1.54 ± 2.30) was notably elevated for normal male cohorts (p = 0.015* and 0.049*) than with participants diagnosed with moderate (19.05 ± 2.04) and severe (10.96 ± 1.35) depression.

The typical age range, which was 38 to 43 years for men, found in this study (**Table 4.13**) did not show any association (p = 0.547) with the acuteness of depression, Alongside the serum IL-6 (p = 0.513) and progesterone (p = 0.0977) levels which had previously associative effects on male participants in the depression group but not in its severity.

Under the stratification (**Table 4.13**) of the females in this study into varying degrees of severity in MDD, it was observed that average age of cohorts also had no direct effect on MDD (p = 0.075), in line with, the serum concentration all the the gonadocorticoids (p = 0.134; 0.773; 0.202). Serum serotonin and IL-6 quantities were once again sought to have significant associations with depression (p = <0.001**; 0.037*). Serotonin quantities

were significantly higher (23.99 ± 1.45) in females grouped into normal than those in moderate (18.62 ± 1.78) or severe (11.22 ± 1.48) depression (**Table 4.13**). On the other hand, serum IL-6 was found increased in moderate (9.55 ± 1.15) than in normal (7.59 ± 1.48) or severe (8.51 ± 1.66) female cohorts.

Table 4.13: Comparison of Variables of Participants Based on Severity of Depression for the Overall Population and Stratified by Sex

	Normal Mean ± SD	Moderate Mean ± SD	Severe Mean ± SD	F	*P*
Overall					
n	60	55	49		

Age (yrs.)	27.95 ± 10.67	31.67 ± 12.33	33.53 ± 12.26*	3.25	**0.041***
Serotonin(ng/mL)	23.99 ± 1.51	18.62 ± 1.86*	11.22 ± 1.45*	34.25	**<0.001****
IL–6(ng/mL)	8.26 ± 3.94	9.48 ± 4.45	8.97 ± 4.68	1.66	0.195
Testosterone(ng/mL)	1.30 ± 2.58	1.25 ± 2.18	1.79 ± 2.74	1.75	0.177
Progesterone (ng/mL)	5.00 ± 1.58	3.49 ± 1.02	2.45 ± 0.51	0.20	0.816
Oestrogen(ng/mL)	0.44 ± 1.10	0.18 ± 0.62	0.11 ± 0.10	1.39	0.252
Male					
n	6	17	14		
Age (yrs.)	43.67 ± 10.21	38.71 ± 9.32	38.14 ± 12.22	0.61	0.547
Serotonin (ng/mL)	25.12 ± 2.24	19.05 ± 2.04	10.96 ± 1.35*	4.80	**0.015***
Interleukin–6(ng/mL)	9.10 ± 5.02	7.80 ± 3.24	7.40 ± 3.86	0.68	0.513
Testosterone(ng/mL)	2.90 ± 3.35	3.18 ± 3.00	5.48 ± 2.66	3.76	**0.033***
Progesterone(ng/mL)	0.24 ± 0.11	0.79 ± 1.73	0.53 ± 1.23	0.02	0.977
Oestrogen(ng/mL)	1.54 ± 2.30	0.31 ± 1.08	0.06 ± 0.01	3.31	**0.0498***
Female					
n	54	38	35	–	–
Age (yrs.)	26.20 ± 9.27	28.53 ± 12.30	31.69 ± 11.95	2.64	0.075
Serotonin (ng/mL)	23.99 ± 1.45	18.62 ± 1.78*	11.22 ± 1.48*	30.09	**<0.001****
Interleukin–6(ng/mL)	7.59 ± 1.48	9.55 ± 1.15*	8.51 ± 1.66	3.39	**0.037***
Testosterone(ng/mL)	1.12 ± 2.46	0.38 ± 0.76	0.32 ± 0.20	2.05	0.134
Progesterone(ng/mL)	5.45 ± 1.65	4.69 ± 1.21	3.21 ± 0.58	0.26	0.773
Oestrogen(ng/mL)	0.31 ± 0.83	0.12 ± 0.21	0.14 ± 0.12	1.62	0.202

SD: Standard Deviation; F: F-statistics; p: p-value.

*P <0.05
**P <0.01
***P <0.001

Source: Author's Laboratory Work, 2024.

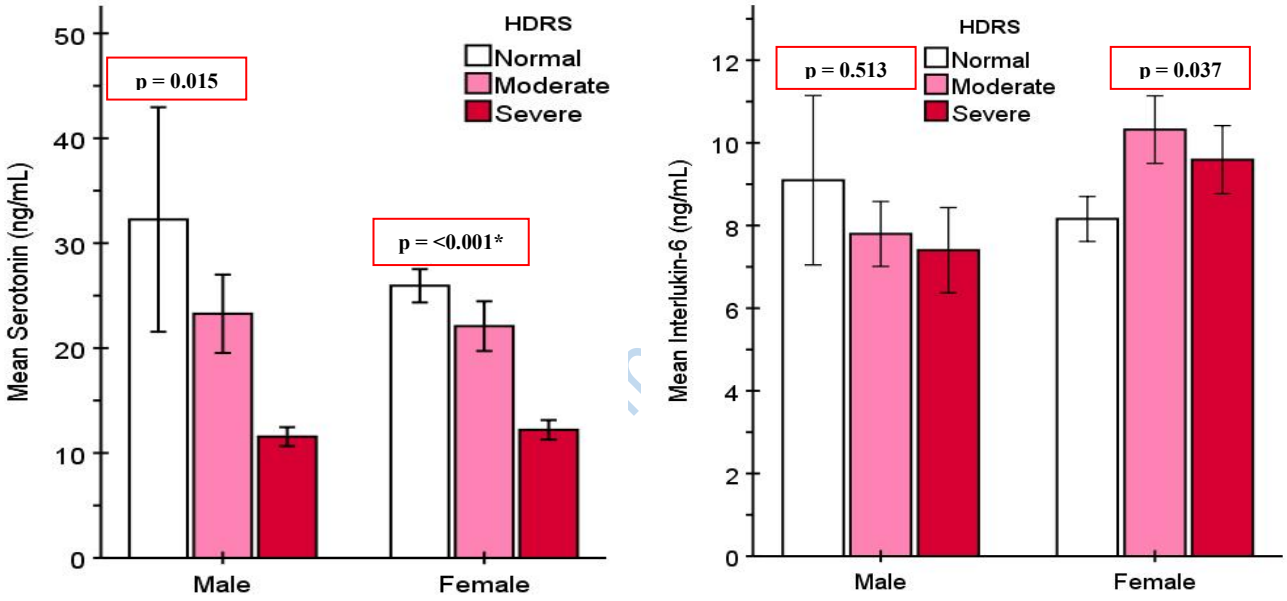


Figure 4.5: Hormonal Distribution in Male and Female Population Based on Severity of Depression

Source: Author's Laboratory Work, 2024.

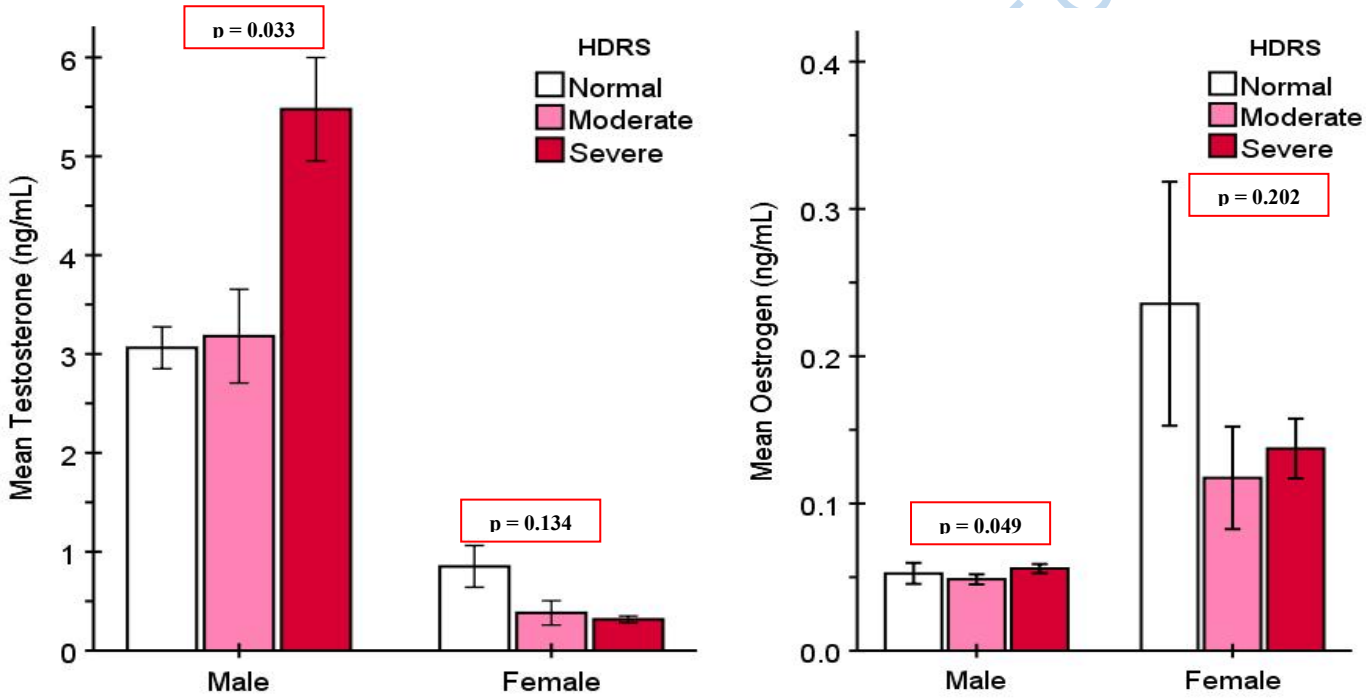


Figure 4.6: Hormonal Distribution in Male and Female Population Based on Severity of Depression

Source:Author's Laboratory Work, 2024.

Table 4.14: Distribution of Serotonin Levels Among the SNPs

The rs8076005 showed an elevated significant association (p = 0.0003**) to low serum serotonin as individuals within this group had on average less than 50ng/mL which are less than than the cut-off range for healthy individuals. It was also observed that a huge number of the participants who had "low serotonin" levels were also found to be homozygous for the G allele. Approximately 94% of the entire population had low serotonin and 50% of them, possessed the GG allele while 31% had the AG allele. rs6354 also showed an association (p = 0.042) with "low serum serotonin" in this polymorph, the the TT genotype had 60% of those with serum serotonin below 50ng/mL. Similarly, 35% of participants GT genotype had low serotonin levels

Table 4.14: Distribution of Serotonin Levels Among the SNPs

	High Serotonin n(%)	Low serotonin n(%)	Total	x^2	*P*
rs8076005			155		
AA	2(20.0)	8(80.0)		6.984	**0.030***
AG	0(0)	49(100.0)			
GG	8(8.3)	88(91.7)			
rs6354			152		
GG	3(14.3)	18(85.7)		2.445	0.295
GT	2(4.4)	43(95.6)			
TT	5(5.8)	81(94.2)			

n:frequency number; %: percentage; x^2:chi square test; P: p-value

*P <0.05
**P <0.01
****P* <0.001

Source: Author's Laboratory Work, 2024.

4.15: Associations between the SNP's Genotypic and Allelic Distribution and Serotonin Levels

The (**Table 4.15**) explored the possible associations between the target SNPs and serotonin levels in the research populace while examine the severity of depression. For rs8076005, individuals with AA genotype were associated with decreased odds of low serum serotonin levels (OR= 0.245; p = 0.004**). While the those with GG genotype were associated with increased odds of low serum serotonin (OR = 4.076, 0.004). Significant associations between rs6354 and participants who have the GG genotype with decreased odds of high serotonin levels (OR = 0.431; p = 0.043) on the other TT twice the increased odds of low serotonin (OR = 2.321; p = 0.043).

Table 4.15: Associations between the SNP's Genotypic and Allelic Distribution and Serotonin Levels

Predictor	Comparison	*OD*	*P*	*95% CI* (LL-UL)
rs8076005				
	AA vs. AG	∞	**0.026***	1.011-593.773
	AA vs. GG	2.75	0.239	0.497-15.208
	AG vs. GG	0	0.052	0.006-1.996
rs6354				
	GG vs. GT	3.583	0.316	0.551-23.294
	GG vs. TT	2.7	0.188	0.591-12.342
	GT vs TT	0.753	1	0.14-4.047

β:type II error rate; SE: Standard Error Z: z-score; p: p-value OR: Odds Ratio; CI: Confidence Interval.

*P <0.05
**P <0.01
***P <0.001

Source:Author's Laboratory Work, 2024.

4.16: Descriptive and Two-Factor ANOVA Statistics of Variables Based on Severity of Depressive for Male Subjects

This analysis that segregated male participants based on their HDRS, further categorized them into < 40 years and ≥ 40 years for each group (**Table 4.16**). Serum serotonin and testosterone levels were found to be significantly raised in HDRS (F = 3.98; 3.96 respectively) and age (F = 5.10; 8.97 respectively) separately but not in unison as co-factors. Highest values of serotonin were recorded for the normal group, in men 40 years and above (38.90 ± 1.66) while men below 40 years in the severe group had least levels (10.72 ng/mL ± 1.00).

Table 4.16: Descriptive and Two-Factor ANOVA Statistics of Variables Based on Severity of Depressive for Male Subjects

	Normal		Moderate		Severe				
	Mean ± SD < 40 yrs.	Mean ± SD ≥ 40 yrs.	Mean ± SD < 40 yrs.	Mean ± SD ≥ 40 yrs.	Mean ± SD < 40 yrs.	Mean ± SD ≥ 40 yrs.	*F* HDRS	*F* age	*F* HDRS–age
n	2	4	11	6	6	8	–	–	–
Serotonin(ng/mL)	10.23 ± 1.35	38.90 ± 1.66	18.62 ± 2.34	19.50 ± 1.41	10.72 ± 1.00	10.96 ± 1.45	**3.98***	**5.10***	2.77
IL-6(ng/mL)	6.56 ± 0.12	10.37 ± 5.96	8.04 ± 3.99	7.35 ± 1.17	6.48 ± 0.92	8.10 ± 5.08	0.38	1.13	0.58
Testosterone (ng/mL)	5.89 ± 1.58	3.80 ± 0.83	1.74 ± 0.63	1.29 ± 0.90	7.41 ± 1.32	3.02 ± 0.44	**3.96***	**8.97***	1.47
Progesterone (ng/mL)	0.24 ± 0.06	0.23 ± 0.14	1.14 ± 2.10	0.15 ± 0.05	0.24 ± 0.13	0.75 ± 1.64	0.02	0.56	0.52
Oestrogen(ng/mL)	0.05 ± 0.01	2.28 ± 2.57	0.45 ± 1.34	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.02	1.77	2.08	2.52

SD: Standard Deviation; F: F-statistics.

Source: Author's Laboratory Work, 2024.

4.17: Descriptive and Two-Factor ANOVA Statistics of Variables Based on Severity of Depressive for Female Subjects

Associations were made in women grouped (**Table 4.17**) according to their HDRS and further into an age strata of < 20 years and ≥ 20 years. Only serotonin and IL-6 levels showed notable significance and this was found only in the HDRS (F = 8.14; 3.18) groups but not in age or as combinative factors

Table 4.17: Descriptive and Two-Factor ANOVA Statistics of Variables Based on Severity of Depressive for Female Subjects

	Normal		Moderate		Severe				
	Mean ± SD < 20 yrs.	Mean ± SD ≥ 20 yrs.	Mean ± SD < 20 yrs.	Mean ± SD ≥ 20 yrs.	Mean ± SD < 20 yrs.	Mean ± SD ≥ 20 yrs.	*F* HDRS	*F* age	*F* HDRS–age
n	26	27	22	24	1	34	–	–	–
Serotonin (ng/mL)	26.30 ± 1.51	22.39 ± 1.35	24.55 ± 1.51	16.60 ± 1.86	8.71 ± 0.00	11.48 ± 1.48	**8.14***	0.39	1.26
IL–6 (ng/mL)	7.76 ± 1.58	7.41 ± 1.38	8.91 ± 1.55	10.00 ± 1.41	13.49 ± 0.00	8.51 ± 1.66	**3.18***	0.69	0.98
Testosterone(ng/mL)	1.75 ± 3.37	0.55 ± 0.92	0.54 ± 1.15	0.31 ± 0.51	0.16 ± 0.00	0.32 ± 0.20	2.05	0.02	0.93
Progesterone(ng/mL)	4.93 ± 1.98	5.92 ± 1.42	5.04 ± 1.34	4.53 ± 1.34	0.13 ± 0.00	3.30 ± 0.58	0.69	0.22	0.74
Oestrogen(ng/mL)	0.37 ± 0.98	0.19 ± 0.36	0.19 ± 0.36	0.09 ± 0.08	0.13 ± 0.00	0.14 ± 0.12	0.43	0.16	0.30

SD: Standard Deviation; F: F-statistics; p: p-value.

Source: Author's Laboratory Work, 2024.

Table 4.18: Inter-Correlation Coefficients Between Variables for Males (above the diagonal) and Females (below the diagonal)

Inter-correlations were drawn to inspect relations in variables in men and women (**Table 4.18**). In men, testosterone levels were found to reduce in men as they age (r = –0.43**; 95%CI = –0.66, – 0.12). Il-6 levels had a directly proportional relationship (increase) with serotonin levels (r = 0.49**; 95%CI = 0.20- 0.71). Oppositely, when serotonin levels were elevated, the testosterone levels diminished (r = –0.49** 95%CI= –0.70, –0.20). Oestrogen and progesterone (r = 0.45**; 95%CI= 0.14, 0.68; r= 0.34* 90.02, 0.60 correspondingly) increased with IL-6 concentrations.

In women, interrelationships were notable. Il-6 levels increased with serotonin levels (r = 0.26** 95%CI = 0.08, 0.42). Testosterone showed an inverse proportionality to the aging (r = –0.25**; 95%CI = –0.41, –0.08) and IL-6 (r = –0.21*; 95%CI= –0.38, –0.03). As age went up their respective levels dropped.

Table 4.18: Inter-Correlation Coefficients Between Variables for Males (above the diagonal) and Females (below the diagonal)

	1.Age	2. Serotonin	3.IL–6	4.Testosterone	5.Progesterone	6. Oestrogen
1. Age	1	0.31 (–0.02, 0.57)	0.18 (–0.15, 0.48)	**–0.43** (–0.66, –0.12)**	–0.02 (–0.35, 0.31)	0.24 (–0.10, 0.52)
2. Serotonin	0.13 (–0.30, 0.05)	1	**0.49** (0.20, 0.71)**	**–0.49** (–0.70, –0.20)**	0.18 (–0.16, 0.48)	**0.33* (–0.10, 0.52)**
3. IL–6	–0.16 (0.02, 0.08)	**0.26** (0.08, 0.42)**	1	–0.28 (–0.55, 0.05)	**0.45** (0.14, 0.68)**	**0.34* (0.02, 0.60)**
4. Testosterone	**–0.25** (–0.41, –0.08)**	–0.05 (–0.23, 0.13)	**–0.21* (–0.38, –0.03)**	1	–0.25 (–0.53, 0.09)	**–0.55*** (–0.74, –0.27)**
5. Progesterone	–0.13 (–0.29, 0.05)	0.10 (–0.08, 0.27)	0.09 (–0.09, 0.27)	0.02 (–0.16, 0.19)	1	0.30 (–0.03, 0.57)
6. Oestrogen	–0.16 (–0.33, 0.02)	–0.14 (–0.31, 0.03)	–0.11 (–0.29, 0.07)	**0.21* (0.03, 0.37)**	**0.36*** (0.20, 0.50)**	1

Pearson's correlations were used, and the correlation coefficient, r (95% CIs), was rounded to 2 decimal places

*P <0.05
**P <0.01
***P <0.001

Source: Author's Laboratory Work, 2024.

Table 4.19: Diagnostic Test Accuracy Measures of Sex and Inflammatory Hormones Levels for Predicting Depression and Varying Severity of Depressive Symptoms In Males

The diagnostic test (**Table 4.19)** measured hormonal levels to recognise cut-off points to predict depression and its prognosis. In men, serum serotonin levels, ≤ 14.13ng/mL (AUC = 0.93; 95%CI = 0.80-0.99) were observed to be the predictive concentration, in essence, men with serotonin levels, than 14 ng/mL may be diagnosed with depression. Testosterone was also found to have significant diagnostic concentration of greater than 4.70 ng/mL (AUC = 0.95; 95%CL = 0.82-1.00).

Moderate depression did not provide any diagnostics cut-off levels. Severe depression in men, on the other hand, was able to propose cut-offs that were similar to overall depression. Serum serotonin levels less than 14ng/mL (AUC = 0.79; 95%CL = 0.63-0.91), testosterone's cutoff for men with sever depression was greater than 4.90ng/mL (AUC = 0.74; 95%CL = 0.57-0.87).

Table 4.19: Diagnostic Test Accuracy Measures of Sex and Inflammatory Hormones Levels for Predicting Depression and Varying Severity of Depressive Symptoms In Males

	AUC (95% CI)	*P*	YI	CP	SN (95% CI)	SP (95% CI)	NPV (95% CI)	PPV (95% CI)
Depression								
Serotonin	**0.93 (0.80, 0.99)**	<0.001	0.89	**≤ 14.13**	100 (82, 100)	89 (65, 99)	98 (99, 100)	91 (72, 97)
Interleukin-6	0.60 (0.43, 0.76)	0.301	0.26	≤ 5.37	26 (9, 51)	94 (73, 100)	55 (48, 62)	83 (39, 98)
Testosterone	**0.95 (0.82, 1.00)**	<0.001	0.79	**> 4.79**	79 (54, 94)	100 (82, 100)	82 (65, 91)	94 (69, 99)
Progesterone	0.52 (0.35, 0.68)	0.873	0.34	> 0.10	95 (74, 100)	33 (13, 59)	86 (44, 98)	60 (52, 68)
Oestrogen	0.51 (0.34, 0.68)	0.928	0.39	≤ 0.06	94 (74, 100)	44 (22, 69)	89 (53, 98)	64 (54, 73)
Moderate								
Serotonin	0.68 (0.50, 0.82)	0.064	0.41	> 14.13	71 (44, 90)	70 (46, 88)	74 (56, 86)	67 (49, 81)
Interleukin-6	0.52 (0.35, 0.69)	0.810	0.15	> 5.25	94 (71, 100)	15 (03, 38)	75 (26, 96)	49 (43, 54)
Testosterone	0.63 (0.45, 0.78)	0.185	0.34	≤ 3.09	59 (33, 82)	75 (51, 91)	68 (54, 80)	67 (46, 83)
Progesterone	0.60 (0.43, 0.76)	0.310	0.30	≤ 0.20	65 (38, 86)	65 (41, 85)	68 (51, 82)	61 (44, 76)
Oestrogen	0.63 (0.45, 0.78)	0.188	0.37	≤ 0.05	47 (23, 72)	90 (68, 99)	67 (56, 76)	80 (50, 94)
Severe								
Serotonin	**0.79 (0.63, 0.91)**	<0.001	0.61	**≤ 14.13**	93 (66, 100)	65 (43, 84)	94 (69, 99)	62 (48, 74)
Interleukin-6	0.59 (0.41, 0.75)	0.411	0.25	≤ 6.17	43 (18, 71)	83 (61, 95)	70 (59, 80)	60 (34, 82)
Testosterone	**0.74 (0.57, 0.87)**	0.004	0.43	**> 4.90**	64 (35, 87)	78 (56, 93)	78 (63, 88)	64 (43, 81)
Progesterone	0.51 (0.34, 0.67)	0.950	0.16	> 0.28	14 (2, 43)	70 (47, 87)	57 (49, 65)	22 (6, 54)
Oestrogen	0.55 (0.38, 0.72)	0.584	0.32	> 0.05	93 (66, 100)	39 (20, 62)	90 (56, 99)	48 (39, 57)

AUC: area under the receiver characteristic curve; YI: Youden index; CP: cutoff point; SN: sensitivity; SP: specificity; NPV: negative predictive value; PPV: positive predictive value; CI: confidence interval.

Source: Author's Laboratory Work, 2024.

Table 4.20: Diagnostic Test Accuracy Measures of Sex and Inflammatory Hormones Levels for Predicting Depression and Varying Severity of Depressive Symptoms In Females

The diagnostic test (**Table 4.20**) in women, measured hormonal levels to recognise cutoff points to predict depression and its prognosis. Serum serotonin levels, ≤ 14.45ng/mL (AUC = 0.90 95%CL = 0.84 - 0.95) were observed to be the predictive concentration. in essence, women with serotonin levels, than 14.45ng/mL may be diagnosed with depression.

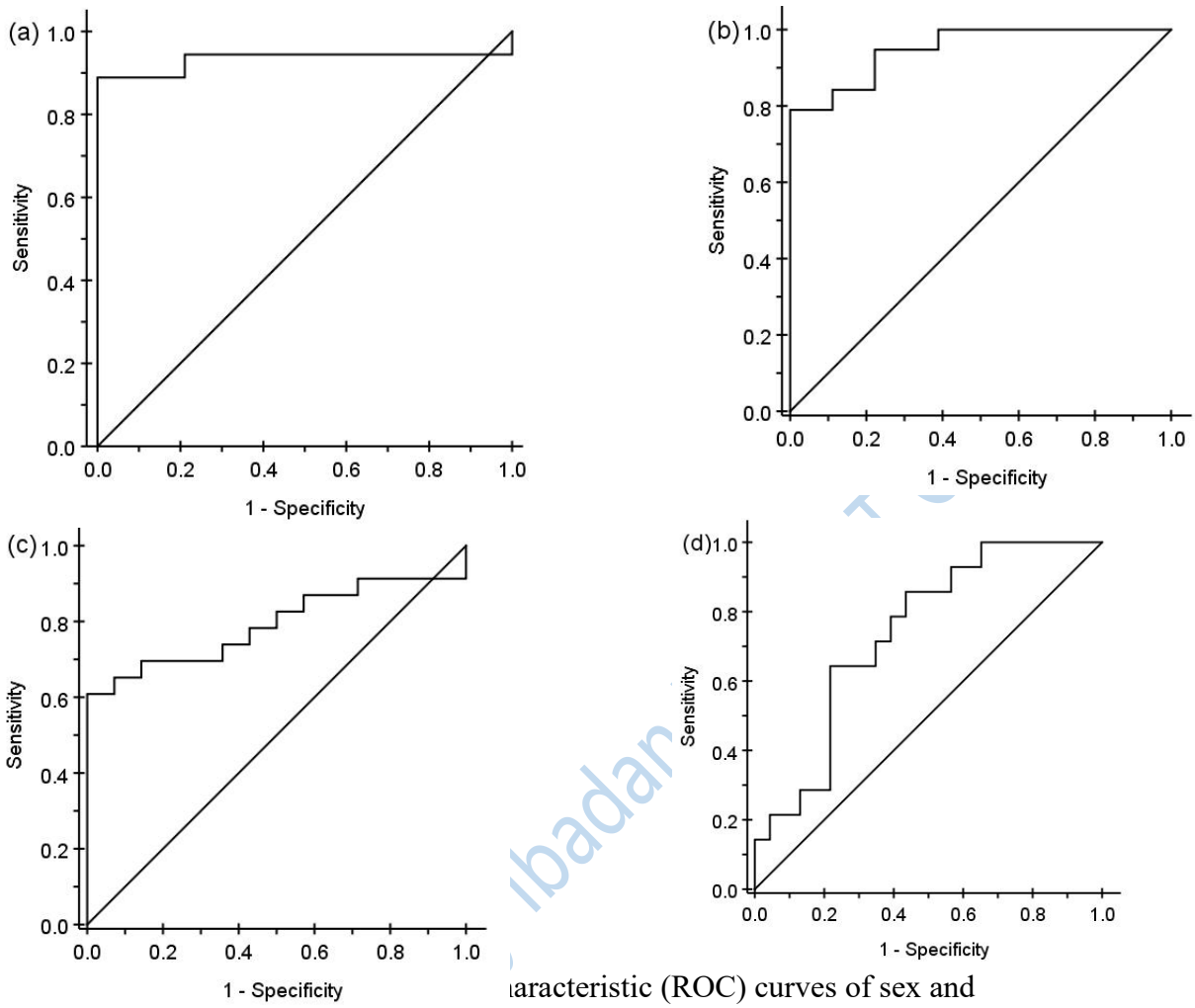
Moderate depression provided diagnostics cut-off levels, interlukin-6 was also found to have significant diagnostic concentration of greater than 7.41ng/mL (AUC = 0.66; 95%CL = 0.57- 0.74). Severe depression in women, on the other hand, was able to propose cutoffs that were similar to overall depression. Serum serotonin levels less than 13.49ng/mL (AUC = 0.85 95%CL = 0.78- 0.91), oestrogen's cutoff for women with severe depression was greater than 0..10ng/mL (AUC = 0.63; 95%CL = 0.54- 0.72).

Table 4.20: Diagnostic Test Accuracy Measures of Sex and Inflammatory Hormones Levels for Predicting Depression and Varying Severity of Depressive Symptoms In Females

	AUC (95% CI)	*P*	YI	CP	SN (95% CI)	SP (95% CI)	NPV (95% CI)	PPV (95% CI)
Depression								
Serotonin	**0.90 (0.84, 0.95)**	<0.001	0.83	**≤ 14.45**	89 (77, 96)	95 (87, 99)	92 (84, 96)	92 (82, 97)
Interleukin-6	0.60 (0.51, 0.68)	0.072	0.32	> 9.77	54 (40, 67)	78 (67, 87)	70 (63, 76)	64 (52, 75)
Testosterone	0.56 (0.47, 0.65)	0.268	0.26	> 0.16	89 (77, 96)	37 (26, 49)	82 (67, 91)	51 (46, 56)
Progesterone	0.50 (0.41, 0.59)	0.956	0.24	> 0.35	30 (18, 44)	47 (35, 59)	47 (40, 55)	29 (21, 40)
Oestrogen	0.58 (0.49, 0.67)	0.125	0.23	> 0.04	93 (82, 98)	30 (20, 42)	85 (67, 94)	50 (45, 54)
Moderate								
Serotonin	0.53 (0.44, 0.62)	0.578	0.17	≤ 14.55	24 (11, 40)	93 (86, 98)	74 (70, 78)	60 (37, 80)
Interleukin-6	**0.66 (0.57, 0.74)**	<0.001	0.34	**> 7.41**	89 (75, 97)	45 (34, 56)	91 (79, 96)	41 (36, 46)
Testosterone	0.59 (0.50, 0.68)	0.091	0.27	≤ 0.35	89 (75, 97)	37 (27, 48)	89 (76, 96)	38 (33, 42)
Progesterone	0.55 (0.46, 0.64)	0.319	0.14	> 0.13	82 (66, 92)	33 (23, 43)	81 (67, 90)	34 (30, 39)
Oestrogen	0.59 (0.50, 0.68)	0.099	0.23	≤ 9.55	79 (63, 90)	44 (33, 55)	83 (72, 90)	38 (32, 43)
Severe								
Serotonin	**0.85 (0.78, 0.91)**	<0.001	0.69	**≤13.49**	89 (73, 97)	81 (71, 88)	95 (88, 98)	65 (54, 74)
Interleukin-6	0.53 (0.43, 0.62)	0.673	0.22	> 9.77	46 (29, 63)	76 (66, 85)	77 (71, 82)	44 (32, 58)
Testosterone	0.58 (0.48, 0.66)	0.160	0.24	> 0.16	83 (66, 93)	41 (31, 52)	86 (74, 93)	35 930, 40)
Progesterone	0.51 (0.42, 0.60)	0.893	0.20	> 0.35	29 (15, 46)	52 (41, 62)	65 (59, 72)	19 (11, 29)
Oestrogen	**0.63 (0.54, 0.72)**	0.013	0.29	**> 0.10**	57 (39, 74)	71 (61, 800	81 (74, 87)	44 (33, 54)

AUC: area under the receiver characteristic curve; YI: Youden index; CP: cutoff point; SN: sensitivity; SP: specificity; NPV: negative predictive value; PPV: positive predictive value; CI: confidence interval.

Source: Author's Laboratory Work, 2024.



F aracteristic (ROC) curves of sex and inflammatory hormones to identify patients with depression and its severity in males (a) ROC curve of serotonin to diagnose depression in males (sensitivity (SN) = 100%; specificity (SP) = 89%; associated criterion ≤ 14.13 ng/mL; area under the ROC curve = 0.93; P <0.001). (b) ROC curve of testosterone to diagnose depression in males (SN = 79%; SP = 100%; associated criterion > 4.79 ng/mL; area under the ROC curve = 0.95; P <0.001). (c) ROC curve of serotonin to diagnose severe level of depression in males (SN = 93%; SP = 65%; associated criterion > 14.13 ng/mL; area under the ROC curve = 0.79; P <0.001). (d) ROC curve of testosterone to diagnose severe level of depression in males (SN = 64%; SP = 78%; associated criterion > 4.90 ng/mL; area under the ROC curve = 0.74; P = 0.004). Plot of sensitivity vs. 1 – specificity. The 45° (reference) line represents chance as a diagnostic criterion (AUC = 0.5).

Source: Author's Laboratory Work, 2024.

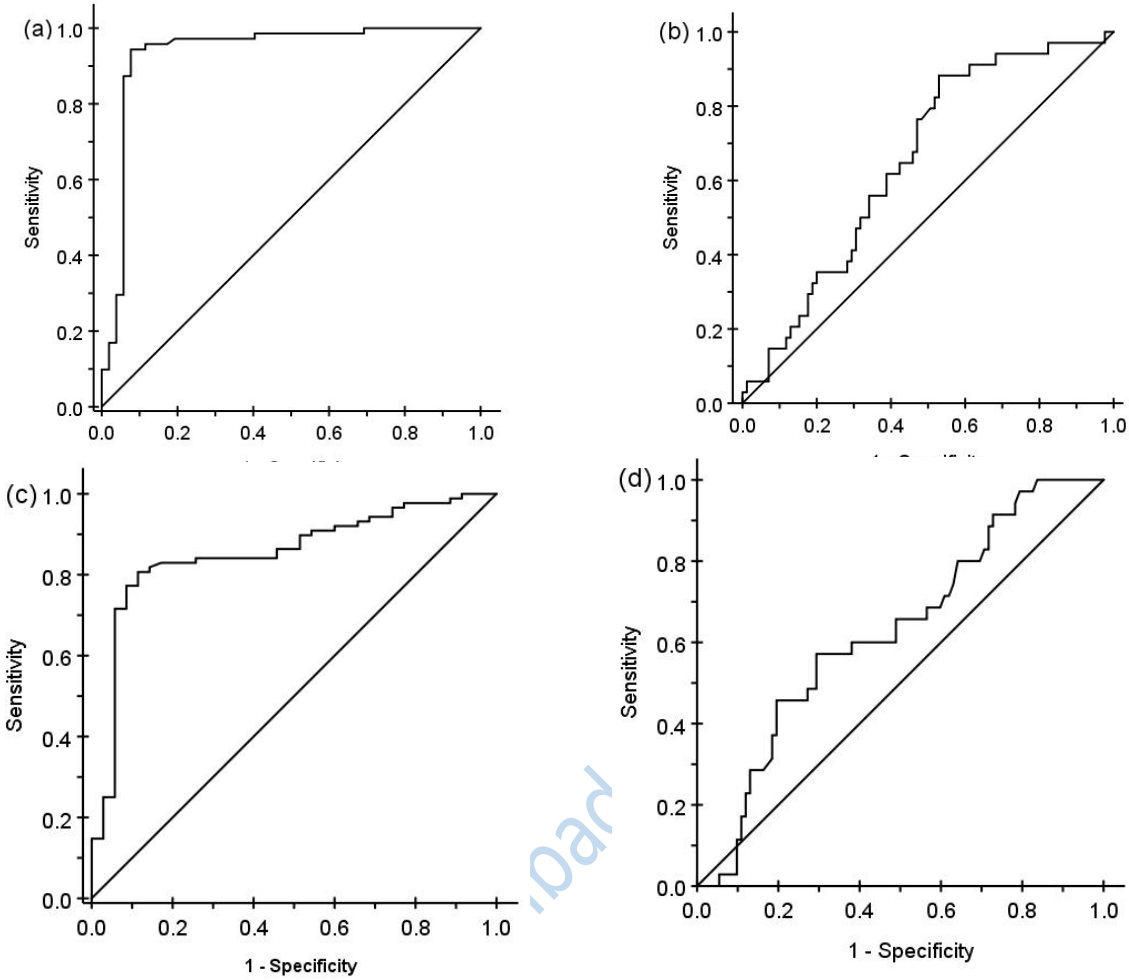


Figure 4.8. a;b: Receiver operating characteristic (ROC) curves of sex and inflammatory hormones to identify patients with depression and its severity in females (a) ROC curve of serotonin to diagnose depression in females (sensitivity (SN) = 89%; specificity (SP) = 95%; associated criterion ≤ 14.45 ng/mL; area under the ROC curve = 0.90; P <0.001). (b) ROC curve of interleukin-6 to diagnose moderate depression in females (SN = 89%; SP = 45%; associated criterion > 7.41 ng/mL; area under the ROC curve = 0.66; P <0.001). (c) ROC curve of serotonin to diagnose severe level of depression in females (SN = 89%; SP = 81%; associated criterion > 13.49 ng/mL; area under the ROC curve = 0.85; P <0.001). (d) ROC curve of oestrogen to diagnose severe level of depression in females (SN = 57%; SP = 71%; associated criterion > 0.10 ng/mL; area under the ROC curve = 0.63; P = 0.013). Plot of sensitivity vs. 1 – specificity. The 45° (reference) line represents chance as a diagnostic criterion (AUC = 0.5).

Source: Author's Laboratory Work, 2024.

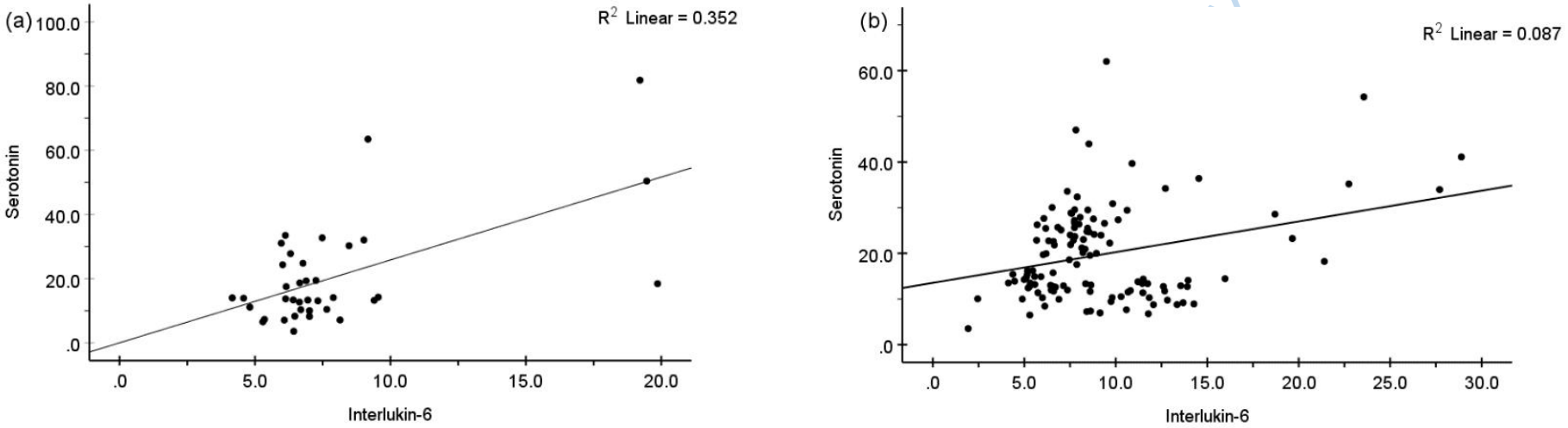


Figure 4.9 a;b: Linear Regression Analyses of Serotonin versus Interleukin-6 (a) Males (b) Females.

Source: Researcher's Laboratory Work, 2024.

4.2 Discussion of Findings

Table 4.1: Study Populations' Enrolment Data

The demographic and baseline clinical data for the 164 participants, split into 73 cases (depressed) and 91 controls (non-depressed). The HDRS classification reveals a significant burden of moderate (33.5%) and severe (29.9%) depression in the case group. Notably, females (77.4%) made up the majority, consistent with global trends in depression prevalence. Stratified age data shows a younger female population (30.7% under 20) and relatively balanced male age distribution. Socio-economic factors such as employment (60.5%) and marital status, as well as health behavior variables like alcohol (37.5%) and smoking (11.2%), provide context for the multifactorial nature of depression. These factors serve as covariates in interpreting biological findings.

The Hamilton Depression Rating Scale (HDRS-17) which categorises depression (normal/clinical remission: 0-7; moderate: 8-19; severity: 20 and above) (**Table 4.1),** grouped the study participants into 60 (36.6%), 55(33.5%) and 49(29.9%) for normal, moderate and severe respectively[1].

Women exhibit a more feminine approach to coping, are more likely to report emotional symptoms, and seek medical attention, which might be another reason for the female majority of depression was surveyed[2]. Also observed was that men, report more severe depression whereas women report milder depression and seeking treatment. Another study proposed, femininity was linked to increased depression levels, even among a group aged. The findings also revealed that women "notice" symptoms, but men "omit" them[3]. This could be one of the factors explaining the disproportional female-male ratio both in control and case admissions.

The study population consisted of 37(22.6%) males and 127(77.4%) females, this results are in accordance to a number of WHO findings, which estimated that women are almost 50% more likely than men to experience depression, which was confirmed by two separate studies, who supported this finding women are known to be disproportionately affected by both diseases, since they are twice as likely to receive a diagnosis and are also more likely to experience mood disorders that are specific to women[4, 5].

Earlier research explained, that marital status was one of the variables linked to depression. Marital status in this study which included being; single, married and divorced/widowed groups divided the population into 97(59.1%), 58(35.4) and 9(5.5) respectively. Two studies proposed that, people who were divorced, widowed, or separated had a higher likelihood of experiencing depression than people who were married or had never married[6].

Distinguished pair of studies, stated that depression and marital status are two of the main elements that affect life satisfaction, which is regarded as a key component for measuring an individual's life quality and subjective well-being[7, 8]. However, more research is necessary to fully understand the mechanisms behind the relationship between depression and marital status. Asian studies noted that, financial and social support are all crucial factors in the mitigation of depression risk among married and single people[9, 10].

A South Korean research, showed employment was linked to better indicators of overall mental health, in contrast to unemployment. The study population had 64(39.5%) of its population employed and 98(60.5%)were unemployed[8].

This study population 68(41.4%) had children and 96(58.6%) said they did not (**Table 4.1**). Smoking and alcohol consumption were taken into consideration as risk factors to depression. 60 (37.5%) drank alcohol, 100 explained they did not, while 4 out of the 164

participants were unaccounted for. Only 18 (11.2%) of the study participants agreed to have been smokers, although the degree of, how often they smoked was not inquired, while 142 (88.8%) had affirmed they did not smoke. The history of the past smoking or drinking habits were also not considered at the time of the enrolment. An interesting fact was explicated; drinking too much alcohol might make depression symptoms worse and make recovery more difficult[10].

In (**figure 4.1)**, the average age of the study population, for women who were diagnosed with depressed was around 30 years old, the control group had a mean age of 26.7 years. On the other hand, the men who were diagnosed with depression were 35.7 years old and those enrolled in the control group had an average were 43 years.

The frequency of the Hamilton Depression Rating Scale among the test and control group, (**figure 4.2)**, shows the distribution of the normal, moderate and severe scale to be 55,18 and 0; 0,19 and 36, 4,12 and 2; and 2,5 and 12 for the women enrolled as control; women diagnosed with depression; men enrolled as control and men diagnosed with MDD.

Table 4.2: Study Populations' SLC6A4 Genomic Data

Genotypic distribution of two SNPs (rs8076005 and rs6354) in the SLC6A4 gene. The 5HTTLPR VNTR segment was not detected in any participants, a novel finding that may reflect population-specific variation or primer mismatch. For rs8076005, GG was the most frequent genotype (59.63%), while for rs6354, TT predominated (54.49%). These distributions highlight a potential genetic pattern distinct from populations where the S allele of 5HTTLPR or other variants are prevalent. The genotypic information lays the groundwork for examining associations with serotonin levels and HDRS scores.

The following findings were observed in various populations; The S allele of the 5HTTLPR-VNTR polymorphism may be linked to an elevated risk of depression by adversely influencing the serotonin absorption rate due to its poor expression, according to a study conducted on a Caucasian sample including 46 MDD patients, aged 19 to 58[11]. In a research conducted in Brazil, it reported that the S allele was the most prevalent in their sample[12]. This finding is comparable to that of a study conducted in an Asian community, which found that over 60% of the MMD samples carried the current allele. Because of its detrimental effects on the serotonin absorption rate, the S allele may thus be linked to the risk of depression[13].

Heterozygous people with the combination of the short and long alleles of 5HTTLPR-VNTR had a significantly greater risk of developing MDD (OR = 1.42, p = 0.02) than people with homozygous genotypes, according to *et al*. Similar outcomes were observed in Mexican populations[14]. In a study conducted with 53 research participants from Mexico, the 5HTTLPR-rs25531 S/La genotype was the most prevalent[15]. Different genetic domains can be found in the same population in Asia. In MDD patients from India, it was discovered the 5HTTLPR-rs25531 SL genotype[16].

In contrast to their non-MDD counterparts, the lifetime MDD and LL genotype participants had larger pericalcarine and lingual volumes, while the lifetime MDD and SL genotype participants had smaller thalamus volumes. However, an alternative study did not find any significant volumetric differences according to 5HTTLPR between the groups with or without lifetime MDD[17]. Because the S allele of 5HTTLPR only raises the risk of depression in people who are under stress found in a study, it shouldn't be broadly applicable, only noticeable in specific contexts, and cohorts with a small sample size[18, 19]. On the other hand, the L allele's enhanced transcriptional activity is regarded protective

against depression, however it has been connected with suicide, nicotine dependency, and attention deficit hyperactivity disorder counterparts[20].

The findings of a further genetic study's analysis provide credence to the link between suicide and the S allele, also known as the SS genotype. Suicidal behaviour is more likely to occur in patients with low-expression 5HTTLPR genes and childhood trauma[21]. Compared to families without a depressed family member, depression is a condition that is more likely to run in families, that have a family with a history of depressive episodes[22]. In a research analysing the 5HTTLPR-VNTR variation in Turkey, the occurrence of the S allele as a risk factor was statistically related with depression ($p < 0.001$), with the S allele present in almost 70% of depressed individuals[19].

However, in a study conducted in Thailand, a strong correlation between the pharmaceutical therapy (reduction of the HAM-D score) and the gene polymorphism[23]. Duloxetine or paroxetine, both SSRIs, did not lower the HAM-D score in depressed individuals with the LL genotype ($p = 0.042$), indicating a limited response to therapy. However, the Asian research mentioned earlier, has revealed that individuals who have responded well to treatment have a high prevalence of the S allele[15]. Pharmacological therapy was positively associated with a decrease in the HAM-D score ($p < 0.0001$) in the Caucasian research, but this was unrelated to the genetic variation[11].

Table 4.3: SNP Phenotype Distribution between the Case and Control

Genotype frequencies of rs8076005 and rs6354 between depressed and control groups. No significant differences were found ($p > 0.05$ for all comparisons), suggesting these SNPs may not independently predict depression risk in this population. However, their relevance may emerge in interaction with biochemical markers or depression severity strata, as explored in subsequent tables.

Table 4.4: SNP Phenotype Distribution between the HDRS Groups

Here, participants are categorized by depression severity (normal, moderate, severe), and their genotypes for rs8076005 and rs6354 are analyzed. Again, no statistically significant associations are observed. However, trends such as higher GG frequency in the severe group for both SNPs hint at a potential dose-response relationship, although not reaching significance (e.g., rs8076005 GG in severe: 36.46%).

Table 4.5: rs8076005 Hardy-Weinberg Distribution across Depression Strata and Control

Hardy-Weinberg analysis reveals that the genotype frequencies of rs8076005 are in equilibrium across all groups ($p > 0.05$). This suggests the absence of strong selection pressure or genotyping errors. Notably, allele G is consistently more frequent than A in all groups, including controls (76%). The lack of deviation supports the reliability of genotypic data and indicates rs8076005 does not directly associate with depression severity.

Table 4.6: rs6354 Hardy-Weinberg Phenotype and Allele Distribution between the Depression Strata and Control

Hardy-Weinberg equilibrium (HWE) investigates for rs6354 across depression severity levels and controls. Interestingly, while the moderate and control groups conform to HWE ($p > 0.05$), the severe depression group significantly deviates ($p = 0.035$), suggesting a potential genotypic shift in more severe cases. The TT genotype remains most frequent across groups, but the higher frequency of the GG genotype in the severe group (20%) compared to controls (10.5%) hints at a potential predictive role of GG for

severity, rather than mere susceptibility. This table supports the hypothesis that rs6354 may act as a severity modifier rather than a primary risk factor.

Here, rs6354, has a significant association with the severe depression (p=0.035). It is deducted that the SNPs do not themselves predispose to depression but may act as a predictive indicator to the the morbidity of those who are diagnosed with depression.

A study proposed, that in the antidepressant group (p = 0.015 and 0.005, respectively) and in the entire sample (p = 0.03 and 0.02, respectively), the SLC6A4 rs8076005 AA genotype and A allele were linked to response rate. A non-significant trend in the same direction was noted in the IPC group. Only in the IPC group did the TPH2 rs11179023 A allele exhibit a weak correlation with symptom improvement. No treatment group's results were impacted by other gene variants[24].

A link between the rs6354 GG genotype and the risk of Alzheimer's disease was demonstrated using recessive model association studies[25]. Literature data points to a potential function of rs6354 in the control of DNA methylation in the SLC6A4 region, which might affect the gene's overall expression, even if in silico research (CpG island finder) did not reveal any methylation focus points. The T > G mutation results in the formation of a branch point site, according to in silico investigation of potential splicing changes associated with this polymorphism. Additionally, the consensus sequence for an exonic splicing silencer (Motif 3—TCTCCCAA) is present in G carriers. However, the rs6354 genotype does not appear to have a major effect on splicing, considering the location of the polymorphism and the magnitude of these changes.

In fMRI studies, greater regional homogeneity (ReHo) in the orbitofrontal areas of females has been reported, which may be responsible for females' higher emotion perception ability[26]. They found that depressive symptoms were positively correlated with

the left medial OFC in females and negatively correlated with the left lateral OFC in males. However, there were no sex-related changes in the right hemisphere[27]. However, a structural whole-brain study examined differences in grey and white matter between the sexes. In addition to healthy controls, they recruited individuals with melancholy sadness; the findings showed a notable decrease in the grey matter volume of the right thalamus in MDD males, which was not seen in their female counterparts[5].

Table 4.7: Distribution of Severity of Depression by Cohort and Sex

This table confirms statistically significant associations between depression severity and both case status ($p < 0.001$) and sex ($p = 0.013$). While control subjects are mostly classified as normal, cases show a gradient from moderate to severe HDRS scores. Females, although more numerous, show a broader severity range, while males tend to present with more severe depression when affected. These findings underscore the importance of sex-specific analysis in depression research and further support the differentiation of cases and controls based on clinical severity.

Table 4.8: Age Distribution of Participants Stratified by Depression and Control

Participants are divided into age and sex subgroups. No significant associations are found in the male group by age or case status. However, a strong association is observed in females ($p < 0.001$), where a higher proportion of depressed females are aged ≥20 years. This aligns with epidemiological data showing that female vulnerability to depression increases with age, potentially due to hormonal fluctuations or life stressors. This sex-specific trend justifies stratifying future analyses by both age and sex.

An association was found between the female age strata (< 20 years and ≥ 20), control and case ($p = <0.001$**). Women are more likely than men to experience depression, but men who suffer from depression are more than three times more likely to die from

depression-related suicide than women[28]. In a cross-sectional study, the gender difference in incidence rates begins at age 12 and peaks during adolescence[5]. This was to accommodate the sex differences found known to occur in the manifestation of depression among men and women.

Table 4.9: Association of Age Distribution and Presence of Children on Case and Control

This table examines whether age and having children differ significantly between cases and controls. Neither variable shows a significant association ($p > 0.05$). While children and age are often explored as potential psychosocial moderators of depression risk, their lack of significance here suggests that biological or genetic factors may play a more dominant role in this population. It is worth noting, however, that nuances like number of children, parenting stress, or support systems were not analyzed and could offer deeper insights.

Meta-analysis investigated, showed bereaved people may have more severe depressive symptoms as they get older[29].

Men did not have a significant correlation between MD and the number of children they had (OR = 1.02, 95%CI = 0.97–1.07), whereas men who resided in cities had a lower odds of having more children (OR = 0.81, 95%CI = 0.71–0.96)[30]. Women had a 4% lower chance of experiencing sleeplessness for every extra kid (OR = 0.96, 95%CI = 0.95–0.96). Every extra kid was also connected to reduced odds of sleep deprivation in men (OR = 0.98, 95%CI = 0.97–1.00).

Table 4.10: Association of Socio-Economic Factors in Case and Control Groups

This table assesses the impact of employment, marital status, smoking, and alcohol consumption on depression status. Employment emerges as a significant protective factor (p = 0.044; OR = 1.869), with unemployed individuals nearly twice as likely to be depressed. Alcohol consumption is inversely associated with depression (p = 0.005; OR = 0.272), suggesting non-drinkers were more likely to be depressed. While this may seem counterintuitive, some literature suggests that light to moderate drinking may be linked to lower depression risk, especially in women. Smoking and marital status show no significant associations here. These findings support a biopsychosocial model, where socio-economic factors interact with biological markers to influence depression outcomes.

Interestingly, employment status was found to be a significant factor influencing against depressive symptoms as the odds of being were significantly reduced, when an individual was employed (OR = 0.029*). In a study, with both men and women more likely to experience depression when they are unemployed. Among women, single status was linked to a higher likelihood of experiencing depressive symptoms than married status, which is consistent with earlier studies[29].

This study (**Table 4.10**) revealed were no associations between marital status or stress related issues in marital relationships (p = 0.601) and the likelihood of being depressed (OR = 1.218; POR = 0.278; 95%CI = 0.637-2.326).

In contradiction to Chinese family panel studies, our investigation supported the relationship between life satisfaction, marital status, and depression symptoms. Middle-aged and older individuals who have never married, separated, or divorced may have poorer life satisfaction, which has been linked to depressive symptoms[31]. This was also supported by a Mexican team in 2019, which observed that those who were never married,

divorced, widowed, or separated had worse prognoses than those who were married among patients with depression[32].

Additionally, depression has been shown to twice the percentage of couples that move from cohabitation or marriage to separation or divorce (HR = 2.0; 95% CI = 1.4 – 2.9 P <0.001). Depression symptoms may have a detrimental impact on marital status and raise the percentage of the population that is single; conversely, being single may encourage the onset of depressive symptoms. The findings of a similar study supported the associations between marital status and depression symptoms, which were in line with earlier research[33].

A depression and schizophrenic research established that both long-time and new smokers had a higher risk of depression and even schizophrenia when they smoked, according to a Mendelian randomisation research including 462,690 people from the UK Biobank[34].

Smoking and alcohol have been associated as predisposing factors to a number of diseases and their outcome. (**Table 4.10**) There were no associations between being a smoker and being depressed (p = 0.162; OR = 0.456; POR = 0.078; 95CI = 0.154-1.346). In contrast, people who drink alcohol have a strong association with not being depressed (p = 0.005**). Additionally it is more likely to be a drinker and be healthy (OR = 0.272; POR = <0.001; 95%CI = 0.135-0.552) than not.

Depression has been linked to high episodic drinking and excessive alcohol consumption which was argued[35]. A complicated relationship between substance use and mental health is revealed by the interaction between alcohol intake and depression. Building on the existing literature on the relationship between alcohol use and depression, it was

discovered that those who drank alcohol in a hazardous manner were more likely to experience depression than those who drank alcohol in a non-hazardous manner[36]. The Korean study furthermore, showing that those who refrained from alcohol intake all together, had a higher risk of depression than light drinkers, their research was able to clarifies the complexities of this association[35].

Similar results were found, when, in-order to investigate the relationship between drinking behaviours and depressive symptoms, the aforementioned study, reported the participants were split up into subgroups and observed that women who drank alcohol moderately (1–4 times per month) were less likely to experience depressive symptoms ($p = 0.024$), while men who began drinking before the age of 19 were more likely to experience depressive symptoms ($p = 0.048$)[10]. The only women who had higher odds of depressive symptoms were those who drank alcohol more frequently (≥ 7 drinks per occasion) ($p = 0.001$). This study did not take into cognisance the frequency of alcohol intake by participants.

Table 4.11: Association of Socio-Economic Factors in HDRS Groups

This table evaluates whether employment status, marital status, smoking, or alcohol consumption correlate with severity of depression. Unlike Table 4.10 (which linked these variables to general depression status), here none of the socio-economic factors show a statistically significant relationship with depression severity (all p-values > 0.05). This suggests that socio-economic conditions may influence the risk of becoming depressed but do not necessarily modulate the progression from moderate to severe depression. For example, although unemployment may increase one's chances of being depressed, it may not directly influence whether the individual ends up moderately or severely depressed.

Smoking or alcohol consumption especially did not show to have any role in the severity of those diagnosed with MDD (p = 0.089 and 0.092 respectively). Which was refuted by a 2019 Korean study on adolescents, which investigated that heated tobacco products (HTP) users who were exposed to second-hand smoke (SHS) at home and in public had a 1.37 (95% CI = 1.10-1.70) and 1.44 (95% CI = 1.18-1.75), respectively, greater risk of suicidal ideation than non-users. Among HTP users exposed to SHS at home, school, and public locations, the probability of suicide attempts was 1.88 (95% CI = 1.37-2.57), 1.45 (95% CI = 1.63-2.00), and 2.21 (95% CI = 1.63-3.00) times higher, respectively[37].

Table 4.12: Comparison of Age and Serological Variables of Participants Across Control and Case Groups

This table provides biochemical comparisons between depressed and control participants, both overall and stratified by sex. The key finding is a significantly lower serotonin level in the depression group (11.22 ± 1.45 ng/mL) compared to controls (25.12 ± 1.58 ng/mL), with a p-value < 0.001. This supports the serotonin hypothesis of depression. These results underscore the interplay between inflammation, sex hormones, and depression.

An additional study, examined that, after starting antidepressant mono-therapy, patients who did not respond well or who had unpleasant side effects were given alternate medicines every three weeks. A 12-week remission was then assessed, which is indicated by a Hamilton Depression Rating Scale (HAMD) score of < 7. After controlling for pertinent covariates, logistic regression models were used to examine the individual and interaction effects of age (as a continuous variable or binary [< 60 vs. ≥ 60 years]) and serum 5-HT level (as a continuous variable or low versus high, based on the median value of 72.6 ng/mL) on the 12-week remission rate. A substantial multiplicative interaction effect and the greatest 12-week remission rates were linked to high 5-HT (≥ 72.6 ng/mL)

and age ≥ 60 years. the study suggested that there was an association between the 12 week antidepressant outcome according to the age of the participants[38].

Patients in a Brazilian research, had a body mass index (BMI) of 36.9 ± 6.2 kg/m2 and an average age of 41.0 ± 10.0 years. Thirty-four percent of patients had depression. Serum serotonin levels in individuals with and without depression did not change significantly (156.4 ± 63.5 vs. 147.7 ± 71.2 ng/mL; p = 0.357)[39].

The Center for Epidemiologic Studies Depression Scale of 20 items (CESD–20) measured depressive symptoms, plasma serotonin levels by ELISA, and the 5–HTTLPR polymorphism was analyzed by PCR. Of the 84 older adults who participated, 39.3% (N = 33) had depressive symptoms, with a median plasma serotonin level of 204.34 ng/mL (SD = 93.88). There was a significant correlation between the CESD–20 scale and plasma serotonin levels (r = –.256; p = 0.019), and low serotonin levels were linked to the presence of depressive symptoms (p = 0.001). Plasma serotonin levels (p = 0.391) and depressed symptoms (p = 0.587) were not linked to the 5-HTTLPR polymorphism. Low plasma serotonin levels are associated with depressive symptoms, but not with the 5-HTTLPR polymorphism in older Mexican individuals[40].

The inflammatory response and its resolution are closely regulated processes, , and the proper cellular responses and resolution of the inflammatory process depend on the balance between pro- and anti-inflammatory systems. However, this equilibrium is gradually ups*et al*s people age, as pro-inflammatory indicators eventually outnumber anti-inflammatory ones (i.e., inflammaging)[41].

Testosterone was significantly lower in depressed males (p < 0.001), suggesting a hormonal contribution to male depressive pathology and Age differences between groups

were not statistically significant overall, but male controls were older than their depressed counterparts (p = 0.032), possibly suggesting age as a resilience factor in men.

According to a different study, the intensity of depressive episodes is correlated with inflammation, and levels of inflammatory markers decline following the recovery of a depressive episode but fall short of those seen in people who have never experienced depression[42]. Remarkably, physical and cognitive deterioration are particularly linked to elevated inflammatory markers including IL-6 and CRP. A pair of studies in 2020, discussed a change in the cellular secretome profile, known as the senescence-associated secretory phenotype (SASP), is one of the most significant features of cellular senescence Numerous pro-inflammatory cytokines, chemokines, extracellular matrix proteases, angiogenic factors, growth factors, cell cycle, and metabolic regulating factors are secreted by senescent cells that accumulate in tissues[43, 44].

Subsequent research using separate cohorts supported preliminary findings made by another pair, showed that a higher SASP index was also linked to global cognitive impairment, particularly executive dysfunction and slower information processing speed went on to demonstrate that young and middle-aged persons with MDD scored higher on the SASP index than people who had never had depression[45, 46.]

Other notable findings: IL-6 was paradoxically higher in females with depression (p = 0.038), but lower in depressed males than controls (p = 0.048), indicating a sex-specific inflammatory response. A 2023 study, recognised higher levels of IL-2 and IL-6 were linked to more severe depression symptoms across the board in the group[47]. Examining each sex independently, we discovered that only boys had a correlation between the intensity of depressive symptoms and IL-2 (rs = 0.38, p =.001), whereas only girls had a correlation with IL-6 (rs = 0.29, p = 0.012). F(1, 146) = 0.927, p = 0.337, the increase of

0.6% in the total variance, indicates that biological sex did not mitigate the impact of IL-6 on the intensity of depression symptoms.

Nonetheless, a straightforward slope analysis revealed a substantial positive correlation between IL-6 and the intensity of depression symptoms in both girls and boys ($p = 0.008$) and not in boys ($p = 0.294$).

According to a unique study, the intensity of depressive episodes is correlated with inflammation, and levels of inflammatory markers decline following the recovery of a depressive episode but fall short of those seen in people who have never experienced depression. Remarkably, physical and cognitive deterioration are particularly linked to elevated inflammatory markers including IL-6 and CRP. it is therefore unclear why the il-6 levels were so raised in the control group[42].

A cancer investigation, found that individuals with severe response to everyday allergens such as; pollen, insect bites reaction to penicillin, copper against the skin, house dust mites and so on, have reduced risk of cancer development in their lifetime[48].

This heightened immune response may mean an ability for cells detection and elimination of tumour mechanisms being highly efficient, or that allergic reposes that involved cytokine release, help stimulate anti tumour immunity or that the allergic reactions have made tumour growth less conducive.

Testosterone levels (**Table 4.12**) showed a statistical increase in men ($p = <0.001$**), contrary to the overall study populace, here, much higher concentrations were found in the individuals diagnosed with depression (5.89 ± 1.48) than in the control (1.74 ± 0.70). Progesterone and oestrogen levels did not show any significance linkages in men as it relates to depression ($p = 0.407$; $p = 0.144$).

The average age in the female population was significantly increased (p = 0.040) in those that were experiencing MDD (30.76 ± 11.54) than in those that were enrolled as control (26.67 ± 10.60).

In "The CHARLS 2018" survey data revealed that middle-aged and older Chinese women had more prominent depression (52.9%), while middle-aged and older Chinese women with chronic illnesses had more severe depressive symptoms (43.6%) with the incidence being two to three times higher than that of men[49]. In line with several long-term studies of the same cohort, differences in depressive symptoms between urban and rural residents were also noted[50, 51]. Personality, genetics, certain chemicals in the body, and metabolic diseases are significant contributors to the increased frequency of depression in women, in addition to the overwhelming pressures placed on them by modern society was proposed by[52, 53].

Serotonin levels (**Table 4.12**) were greatly decreased in depressed females (p =10.72 ± 1.48) and a strong association was detected (p = <0.001**) in comparison to control (25.12 ± 1.48). In contrast, IL-6 levels were raised in females who are depressed (8.51 ± 1.58) than that in the control (7.24 ± 1.45) significantly (p = 0.038). Observed testosterone, progesterone and oestrogen levels were not detected to have any substantial links to depressed or control cohorts (p = 0.351; 0.462; 0.374 respectively).

Table 4.13: Comparison of Variables of Participants Based on Severity of Depression (Overall and by Sex)

This table investigates hormonal and inflammatory markers across depression severity (normal, moderate, severe). The following are significant findings:

Serotonin levels declined with increasing severity ($p < 0.001$), reinforcing its utility as a biomarker for depression progression; Testosterone significantly increased in severely depressed males ($p = 0.033$), possibly reflecting a compensatory mechanism or an stress-related rise; Oestrogen levels dropped significantly in severely depressed males ($p = 0.0498$), again highlighting sex-based endocrine variation; In females, IL-6 increased in the moderately depressed group ($p = 0.037$), which might represent a pro-inflammatory phase before adaptive burnout in severe cases; Age increased with severity overall ($p = 0.041$), suggesting cumulative psychosocial or biological burden. **Table 4.13** emphasizes how biomarkers track not only presence but also intensity of depressive symptoms, especially serotonin and inflammatory markers.

Low oestrogen levels are thought to be the main risk factor for female depression since several research conducted in the last few decades have demonstrated the important role oestrogen plays in neuroprotection and anti-inflammation[57]. A well-known illustration of this idea is the fact that postpartum and perimenopause, two times when women are more vulnerable to depression, are frequently marked by a marked drop in blood oestrogen levels. According to a lower oestrogen level is negatively associated with oxidative stress and vascular wall inflammation, which causes a number of painful perimenopausal symptoms. Conversely, Albert and Newhouse (2019), stated that exogenous oestrogen supplementation can successfully alleviate menopausal symptoms and depression symptoms in as many as 30% of patients Exogenous oestrogen supplementation, on the other hand, has been shown to successfully alleviate menopausal symptoms and depression symptoms in as many as 30% of cases.

According to popular medical articles, elevated oestrogen levels can result in symptoms including sadness, erectile dysfunction, and infertility. In general, little is known about

specialised research that addressed male depression in relation to hormonal balance and its serotonin pathways[55, 56].

Table 4.14: Distribution of Serotonin Levels Among the SNPs

This table explores the relationship between serotonin levels and the genotypes of rs8076005 and rs6354. Both SNPs show strong, significant associations with low serotonin levels ($p < 0.001$): Individuals with the rs8076005 AG and GG genotypes are overwhelmingly represented in the low serotonin group; Similarly, rs6354 GT and TT genotypes correlate with low serotonin, with TT having the highest representation (53.3%).

These findings support a genotype–phenotype linkage, where specific SLC6A4 variants correspond to reduced serotonin bioavailability, potentially via decreased transporter function or altered expression.

It is important to note that the rs8076005 and rs6354 are both point polymorphs on the SLC6A4 gene which are responsible for the modulation of the serotonin. Therefore, possible associations to asses if these SNPs had causative effects on not just average but on “high” or “low” serotonin levels within our study participants (**Table 4.14**).

The rs8076005 showed an elevated significant association ($p = 0.0003$**) to low serum serotonin as individuals within this group had on average less than 50ng/mL which are less than than the cut-off range for healthy individuals. It was also observed that a huge number of the participants who had “low serotonin” levels were also found to be homozygous for the G allele. Approximately 94% of the entire population had low serotonin and 50% of them, possessed the GG allele while 31% had the AG allele. rs6354 also showed an association ($p = 0.042$) with “low serum serotonin” in this polymorph, the

the TT genotype had 60% of those with serum serotonin below 50ng/mL. Similarly, 35% of participants GT genotype had low serotonin levels.

Table 4.15: Associations Between the SNP's Genotypic and Allelic Distribution and Serotonin Levels

This logistic regression table confirms and quantifies the associations between SNP genotypes and serotonin levels: rs8076005: The AA genotype is significantly protective (OR = 0.245, p = 0.043), while GG is a risk factor (OR = 2.573, p = 0.004).

rs6354: The GG genotype is also protective (OR = 0.23, p = 0.007), and TT is significantly associated with low serotonin (OR = 2.321, p = 0.045). The severity of depression also independently predicts serotonin levels, particularly the severe group (OR = 13.46, p = 0.002). This table strongly supports a gene-serotonin-depression axis, where genetic variants modulate serotonin concentration and, in turn, influence depressive severity.

This could hint at the Homozygous allele 2 (recessive allele) which are the in both the rs8076005 and rs6354 are able to deregulate the assembly of serum serotonin pathways. Also may bring to light, (**Table 4.14**) that individuals who were mere carriers of the G allele at the rs8706005 may not be protected against the susceptibility or severity of depression.

Genetic research observed that rs8076005 (A/G), five other SNPs, and BDI were strongly correlated (P ≤0.008), and that one SNP was borderline linked (rs12150214, P = 0.017). Three of these seven SNPs—rs25528, rs6354, and rs8076005—were likewise strongly (P<0.008) linked to IL-6, whereas the remaining four were only marginally (P = 0.009~0.025) related. Higher BDI scores and IL-6 levels were seen in participants

carrying one copy of each of these seven SNPs' minor alleles. Additional bivariate analysis showed that the SLC6A4 gene might account for around 10% of the relationship between BDI and IL-6[57].

Interactions between burnout and Chinese health workers in a hospital reported, most relationships between the three burnout subscores and the job stress score or the six stressor scores were significant (all $p < 0.05$). A statistically significant interaction between 5-HTT rs6354 and job stress on burnout was found ($F = 5.08$, $df = 2, 369$, $p = 0.007$), but no significant main impact of the 5-HTT rs6354 genotype on burnout symptoms was found. G allele carriers experienced burnout at a considerably greater level than TT homozygotes in the low stress group ($F = 11.60$, $df = 1, 48$, $p < 0.001$). Conversely, G allele carriers showed a considerably lower degree of burnout than the TT homozygote in the high stress group ($F = 3.86$, $df = 1, 103$, $p = 0.025$)[58].

Age stratification was carried out as described (**Table 4.1** and **Table 4.8**) not only to investigate the effect of chronology and depression but also because the adversity of illnesses are said to increase as humans get older and cellular efficiency[30]. It was also reported that men over the age of 40, are more likely to commit suicide when diagnosed with depression, in light of this, it was important to observe if there were any hormonal imbalances with age.

Table 4.16: Descriptive and Two-Factor ANOVA of Variables Based on Severity of Depression for Male Subjects

This table explores the interaction between age strata (<40 vs. ≥40) and HDRS depression severity on biochemical variables in males using two-factor ANOVA. Key findings include: Serotonin levels significantly decreased with increasing depression severity (F

HDRS = 3.98*) and were also affected by age (F age = 5.10*), suggesting age and severity independently influence serotonin in men.

Testosterone also showed significant associations with both depression severity (F HDRS = 3.96*) and age (F age = 8.97*), supporting its role as a sex-specific modulator of depression. Other hormones (IL-6, progesterone, oestrogen) did not show significant effects, though oestrogen levels were extremely low across the board in men.
This table underscores the dual influence of age and depression severity on key hormonal pathways in males, with serotonin and testosterone emerging as critical biomarkers.

Testosterone levels, whereas, documented its highest volumes in men who were categorised to have severe depression and were below 40 years (7.41 ng/mL ± 1.32). Younger men who were diagnosed with severe depression had the highest levels of testosterone across all groups.

Table 4.17: Descriptive and Two-Factor ANOVA of Variables Based on Severity of Depression for Female Subjects

Similar to Table 4.16, but focused on females stratified by age (<20 vs. ≥20). Main findings: Serotonin levels significantly declined with increasing severity (F HDRS = 8.14*), but age did not significantly influence serotonin; IL-6 levels were associated with HDRS severity (F = 3.18*), suggesting a strong inflammatory component in female depressive pathology.

Other hormones (testosterone, progesterone, oestrogen) did not significantly vary across age or depression severity, although a trend was visible in oestrogen.
This supports the idea that inflammatory signaling plays a more prominent role in females, while hormonal influences are more pronounced in males.

Again the trend of high IL-6 levels protecting against depression (as seen in **Table 4.12** especially in men) or its causative agents (which would include low serotonin) was seen to once more be in play(serotonin levels showed their least concentrations here). could it also be deduced that if increased IL-6 values were acting as a protective biomolecule and testosterone was found to be reduced in the presence of increased IL-6 that elevated testosterone may be playing as significant stressor to determining if an individual is not protected against MDD **(Table 4.12.**) and of reduced testosterone which is expected as men age, why are older men more likely to commit suicide[59]**.**

In contrast to the finds of this study, Serum high-sensitivity (hs-) CRP was evaluated in 64 healthy controls and 178 MDD patients recently. The patients were classified into two groups: those with hs-CRP below 3 mg/L (low-CRP; 53 males, 72 females) and those with high-CRP over 3 mg/L (high-CRP; 19 men, 34 females). Progesterone, sex-hormone binding globulin (SHBG), interleukin-6, testosterone, 17-β-estradiol (E2), progesterone, follicle-stimulating and luteinizing hormones, and the testosterone-to-E2 ratio (T/E2), free androgen and estradiol indexes (FAI, FEI), and testosterone secretion index were also examined[59].

Males with high CRP had lower testosterone than controls ($p = 0.001$), while those with low CRP had greater FEI ($p = 0.015$), lower testosterone ($p = 0.013$), and T/E2 ($p < 0.001$). In females, SHGB levels were lower in high-CRP patients than in low-CRP patients ($p = 0.034$) and controls ($p = 0.033$). The Benjamini-Hochberg FDR correction did not affect the variations in male testosterone, T/E2 ratio, or FEI levels. In linear regression analysis, CRP levels were predicted by SHBG ($\beta = -0.628$ $p = 0.009$, $R2 = 0.172$ $p = 0.003$) in female patients and testosterone ($\beta = -1.069$ $p = 0.033$) in male patients ($R2 = 0.252$ $p = 0.002$).

Table 4.18: Inter-Correlation Coefficients Between Variables for Males and Females

This correlation matrix reveals several important sex-specific biochemical relationships: In males, serotonin correlates positively with IL-6 (r = 0.49**) and negatively with testosterone (r = –0.49**), suggesting inverse regulation between mood neurotransmitters and androgens.

Age and testosterone are negatively correlated (r = –0.43** in males), consistent with age-related hormonal decline. In females, IL-6 is positively correlated with serotonin (r = 0.26**) and oestrogen (r = 0.34*), suggesting linked inflammatory and hormonal changes in women. These patterns emphasize the sex-dependent regulation of mood-related biomarkers, reinforcing the need for sex-specific approaches in research and treatment.

Table 4.19: Diagnostic Test Accuracy for Hormones in Predicting Depression and Severity in Males

This table presents ROC curve metrics evaluating how well different hormones predict depression and its severity in males. Serotonin and testosterone show excellent predictive value for diagnosing depression (AUC = 0.93 and 0.95 respectively, both $p < 0.001$), with high sensitivity and specificity. In severe depression, serotonin remains a strong marker (AUC = 0.79) and testosterone is moderately predictive (AUC = 0.74, p = 0.004). IL-6, progesterone, and oestrogen have poor AUCs (< 0.6), making them weak predictors in males. This indicates that serotonin and testosterone are robust male-specific biomarkers for detecting depression and stratifying its severity.

The diagnostic test (**Table 4.19)** measured hormonal levels to recognise cut-off points to predict depression and its prognosis. In men, serum serotonin levels, ≤ 14.13ng/mL (AUC = 0.93; 95%CI = 0.80-0.99) were observed to be the predictive concentration, in essence, men with serotonin levels, than 14 ng/mL may be diagnosed with depression.

Testosterone was also found to have significant diagnostic concentration of greater than 4.70 ng/mL (AUC = 0.95; 95%CL = 0.82-1.00). Moderate depression did not provide any diagnostics cut-off levels. Severe depression in men, on the other hand, was able to propose cut-offs that were similar to overall depression. Serum serotonin levels less than 14ng/mL (AUC = 0.79; 95%CL = 0.63-0.91), testosterone's cutoff for men with sever depression was greater than 4.90ng/mL (AUC = 0.74; 95%CL = 0.57-0.87).

Table 4.20: Diagnostic Test Accuracy for Hormones in Predicting Depression and Severity in Females

Female-focused ROC analysis shows: Serotonin has excellent diagnostic utility (AUC = 0.90, $p < 0.001$) for depression, confirming its status as a universal biomarker across sexes; IL-6 is a moderate predictor for moderate depression (AUC = 0.66, $p < 0.001$); Other hormones (testosterone, progesterone, oestrogen) perform poorly overall (AUCs mostly ~0.5–0.6), except oestrogen for severe depression (AUC = 0.63, $p = 0.013$).

In females, inflammatory markers show greater predictive relevance, especially IL-6 and oestrogen in moderate-to-severe cases. This supports a distinct female inflammatory-depression phenotype.

Receiver Operating Characteristic (ROC) analysis confirmed the utility of serotonin and testosterone as powerful diagnostic biomarkers for depression in males, while serotonin and IL-6 were more predictive in females. These findings collectively support the presence of sex-specific biomolecular profiles of depression, which may have diagnostic and therapeutic relevance.

These findings underscore the importance of integrating genetic, biochemical, and demographic variables to understand the complex etiology of MDD, particularly within

underrepresented populations such as Nigerians. Future studies should consider longitudinal designs and expand the genomic analysis to include broader polymorphic regions to further elucidate causal mechanisms.

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Chapter Five

Conclusion

5.1 Summary of Findings

Looking at Depression as a clinical disease, not written off as a “mood swing” from over “expressive or repressive” individuals but perceived as an actual fault of the brain’s neurotransmitters, or the fate of bearing a disadvantaged gene or even the disadvantaged sex, Similar to disease such as: Alzheimer's or Parkinson’s whose effects also degenerate over time; in the case of Alzheimer's induced memory loss while in that of the research matter, death by suicide. It has become not only apparent to de-stigmatize but to also study genetic and environmental factors that can lead to patient deterioration.

This study provides valuable insights into the relationship between genetic factors, serotonin levels, inflammation, sex differences (both cultural and hormonal) on depression's occurrence and severity, with particular focus on ageing. The following are an overall can be drawn based on the comprehensive analysis of the study's findings:

1. **Influences from Demographics and Lifestyle:** The study sample is primarily female (77.4%), and the average age of the participants is substantially over 25, indicating that depression severity is higher in these subgroups. The high rate of depression among females of a certain age and the unequal distribution of sexes underscore the need for focused mental health interventions for this population. Lifestyle factors such as alcohol consumption (which showed protective associations) and smoking (showed no significant association) were in line with previous research that links these activities to mental health outcomes, but they were not shown to be possible aggravating factors for depression in our study. Employment also proposed beneficial factors as odds of depression reduced when participants were employed.

2. **Genetic Associations (rs6354 and rs8076005 SNP):** The genetic analysis revealed that individuals who were homozygous for allelle2/alternative allele for both SNPs all showed reduced levels serotonin across the test group and control which would directly have disadvantageous effects in the face of depression occurrence. This could hint at the the allele2 in both the rs8076005 and rs6354 had the ability to deregulate the assembly of serum serotonin pathways. This suggests that genetic factors modulate depression susceptibility and severity through serotonin pathways.

On the opposite spectrum, Individuals with the AA (rs8067005) and GG (rs6354) both have the highest average serotonin levels across all groups.

The idea that genetic predispositions, such as polymorphisms, play a crucial role in affecti ng serotonin regulation and depression severity is further supported by the substantial corr elation found between rs6354 and severe depression.

3. **Serotonin Deficiency as a Biological Marker**: The substantial decline in serotonin levels in depressed people relative to controls is a consistent observation across all severity categories and both sexes. This biological marker supports the idea that serotonin dysregulation plays a factor in the pathophysiology of depression by showing a high correlation with depression severity, especially in severe instances. The use of serotonin as a critical biomarker for the diagnosis and follow-up of depression, particularly in severe instances, is supported by the fact that serotonin levels sharply decrease as depression severity increases. Diagnostic values from this study, can also be adopted in diagnosis of depression (levels lower than 14ng/mL).

4. **Sex-Specific and Age-Specific Findings:** Sex differences in depression severity were observed, with females being more likely to experience moderate depression compared to males. The findings suggest that age and sex interact with serotonin regulation. As younger individuals exhibit higher baseline serotonin and testosterone levels, which decline steeply with increasing severity of depression in males and females. The interaction between age and testosterone levels in males, particularly in severe depression, points to the potential influence of ageing on biochemical disruptions in depression. Further research is needed to explore these age- and sex-specific differences in more detail.

5. **Inflammation:** In this study, IL-6 levels were repeatedly found to be increased in females suffering from depression, with a large number of this particular sex being diagnosed with moderate depression. It could hint at an undiscovered protective nature of

il-6 especially in women who developed depression from falling into more critical episodes and less reported cases of suicide

6. **Synergistic Associations:** There were certain levels of interdependency between some hormones. One important dependency, was between that of serotonin and testosterone levels especially in men. Since increased levels of average testosterone was found in severe cases of depression with men who were below 40 years old, with this same group suffering from the lowest levels of serotonin recorded amongst men for this study population. It is clear that this study observed a "unicorn" finding for depression in men.

5.2 Conclusion

This study has provided valuable insights into the complex relationships between genetic factors, serotonin levels, gonadocorticoids, inflammation, and demographic characteristics in the severity of depression among patients from Nigeria. The findings suggest that genetic variations in the SLC6A4 gene, particularly the rs6354 and rs8076005 SNPs, play a significant role in modulating depression susceptibility and severity through serotonin pathways. Furthermore, the study highlights the importance of sex-specific and age-specific factors in depression, with females and older adults being more vulnerable to depression.

The study's findings have significant implications for the diagnosis, treatment, and management of depression in Nigeria. The identification of genetic markers and biological markers, such as serotonin levels, could facilitate the development of personalized treatment approaches. Moreover, the study's emphasis on the interplay between genetic, biochemical, and demographic factors underscores the need for a holistic approach to addressing depression.

In summary, this study contributes significantly to the understanding of depression in Nigeria, highlighting the complex interplay between genetic, biochemical, and demographic factors. The findings have important implications for the diagnosis, treatment, and management of depression, and underscore the need for further research into this complex and debilitating condition.

5.3 Recommendations

Clinical Consequences

1. **Serotonin-based therapies**: For those with depression who have low serotonin levels, take into consideration serotonin-based therapies, such as selective serotonin reuptake inhibitors (SSRIs).
2. **Customised treatment strategies:** Develop personalised treatment strategies based on individual traits, such as sex-specific variations and genetic variations.
3. **Inflammatory marker-based therapies**: For those with depression who have raised inflammatory markers, take into consideration inflammatory marker-based treatments, such as anti-inflammatory drugs.

4. **Increased awareness and education:** To mitigate the stigma surrounding depression and encourage early intervention, raise knowledge and educate people about depression, its symptoms, and available treatments.

Implications for Policy

1. **More financing for mental health research**: To better understand the causes and effects of depression and to provide more potent therapies, more money should be allocated to mental health research.

2. **Better access to mental health services:** To lessen gaps in mental health outcomes, improve access to mental health services, especially for underprivileged groups.

3. **Lessening stigma and raising awareness**: To encourage early intervention and treatment-seeking behaviour, lessen stigma and raise knowledge about mental health conditions, particularly depression.

5.4 Contribution to Knowledge

The study's potential contributions are as follows:

Contributions in Theory

1. **Promotion of the serotonin theory of depression**: The work supports the serotonin hypothesis by demonstrating how serotonin dysregulation contributes to the pathophysiology of depression.

2. **Genetic tendency to depression**: By identifying particular genetic variations (rs6354 and rs8076005) linked to depression severity and susceptibility, the study advances our knowledge of the genetic foundation of depression.

Contributions from Empiricism

1. **Sex-specific and age-specific variations in depression**: The study shows that depression severity, serotonin levels, and testosterone levels vary by sex and age, underscoring the significance of taking these variables into account when studying and treating depression.

2. Inflammatory markers in depression: The study indicates that females with depression had higher levels of IL-6, which may indicate that inflammation has a part in the pathophysiology of depression.

Practical Contributions

1. Creation of a possible biomarker for depression: According to the study, serotonin levels may be a biomarker for the diagnosis and severity of depression, which may guide the creation of more precise diagnostic instruments and therapeutic approaches.

2. Consequences for individualised treatment strategies: The results of the study on inflammatory markers, age and sex-specific variations, and genetic predisposition in depression point to the possibility of individualised treatment plans catered to each patient's unique requirements and traits.

5.5 Suggested Areas for Future Research

Future research should focus on replicating these findings in larger and more diverse populations, as well as exploring the potential therapeutic applications of these findings. Additionally, studies examining the impact of environmental and socio-cultural factors on depression in Nigeria would provide a more comprehensive understanding of this complex condition.

Future research could investigate the following areas:

1. **Replication studies**: Replicating of the findings of this work especially in larger independent samples to confirm the absence of the 5HTTLPR CNV in people of Nigerian descent; the prospective qualities of increased IL-6 protection in women with depression; and the significant effect of increased testosterone on men with severe depression found in this study.

2. **Marital satisfaction**: this is a factor that should be observed especially in the different sexes instead of the blanket "marital status".

3. **Functional Studies**: the associations that could possibly be at work between the rs6354(found in the promoter region of this gene) are important in gene expression studies, not forgetting rs80766005's role in mRNA stability and translation.

4. **Other Gene-Environment Interactions**: such as early onset childhood stress and trauma which impact a number of psychiatric disorders.

5. **Further studies on the biological mechanisms:** underlying the age- and sex-specific differences in serotonin regulation in the brain through brain imaging and depression severity in a Nigerian population, as this could inform the development of more effective, tailored therapeutic approaches.

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Appendices

Appendix 1: **Ethical Clearance Approval Letters**

National Health Research Ethics Committee of Nigeria (NHREC)

Promoting Highest Ethical and Scientific Standards for Health Research in Nigeria

Federal Ministry of Health

NHREC Protocol Number NHREC/01/01/2007- 30/08/2024
NHREC Approval Number NHREC/01/01/2007-01/09/2024
Date: 1st September, 2024

Re: Role of Serotonin Levels, Gonadocorticoids, Inflamation and SLC6a4 Gene (5HHTLPR VNTR, rs6354 & rs8076005) On the Severity of Depression in Patients from three Geo-Political Zones of Nigeria.

Health Research Committee assigned number: NHREC/01/01/2007

Name of Student Investigator: Obianuju Ibifuro Ojikah
Address of Student Investigator: Faculty of Basic Medical and Applied Sciences
Lead City University, Ibadan
Email: obianujuojikah@gmail.com

Date of receipt of valid application: 30/08/2024
Date when final determination of research was made: 01-09-2024

Notice of Full Committee Review and Approval

This is to inform you that the research described in the submitted protocol, the consent forms and other participant information materials have been reviewed and given full committee approval by the National Health Research Ethics Committee.

This approval dates from 01/09/2024 to 31/08/2025. If there is delay in starting the research, please inform the HREC so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside of these dates. *All informed consent forms used in this study must carry the HREC assigned number and duration of HREC approval of the study.* **If this is a multi-year research, endeavour to submit your annual report to the HREC early in order to obtain renewal of your approval and avoid disruption of your research.**

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the HREC. No changes are permitted in the research without prior approval by the HREC except in circumstances outlined in the Code.

The HREC reserves the right to conduct compliance visit to your research site without previous notification.

Signed

Professor Richard A Adegbola, MSc, PhD (Dundee), FIBMS (UK), FRCPath (London) FRCP (Hons, London), FAS, FAAS, FAMedS.
Chairman, National Health Research Ethics Committee of Nigeria (NHREC)

Author's Ethics Certificate From the Federal Ministry of Health.

Zertifikat
Certificat
Certificado
Certificate

TRREE

Promouvoir les plus hauts standards éthiques dans la protection des participants à la recherche biomédicale
Promoting the highest ethical standards in the protection of biomedical research participants

Certificat de formation - Training Certificate

Ce document atteste que - this document certifies that

Obianuju Ojikah

a complété avec succès - has successfully completed

Training in the Nigerian National Code for Health Research Ethics

du programme de formation TRREE en évaluation éthique de la recherche
of the TRREE training programme in research ethics evaluation

Registration Number: NHREC/TR/08/10/2013A

Release Date: 2024/06/22

CID : J67FJU9BC

Professeur Dominique Sprumont
Coordinateur TRREE Coordinator

This training is supported by Fogarty International Center and West African Bioethics

Ce programme est soutenu par - This program is supported by :

European and Developing Countries Clinical Trials Partnership (EDCTP) (www.edctp.org) - Swiss National Science Foundation (www.snf.ch) - Canadian Institutes of Health Research (http://www.cihr-irsc.gc.ca/e/2891.html) - Swiss Academy of Medical Science (SAMS/ASSM/SAMW) (www.samw.ch) - Commission for Research Partnerships with Developing Countries (www.kfpe.ch)

[REV : 20151109]

NATIONAL HOSPITAL

(Established by Act No 36 of 1999).

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AG. DIRECTOR OF CLINICAL SERVICES/CMAC
Dr. Badejo Olawale A., MBBS, Lagos, FWACS

NHA/ADMIN/236/V.VII/

17th April, 2023

RE: "EFFECTS OF SLC6A4 GENE, LACTOBACTERIA AND GONATOCORTICOIDS ON THE SEROTONIN LEVELS AND INFLAMMATION IN INDIVIDUALS SUFFERINGS FROM DEPRESSION" NHA/EC/019/2023

Health Research Ethics Committee (HREC) Assigned number:	NHA/EC/019/2023
Name of Principal Investigator:	**Obianuju Ibifuro Ojikah**
Address of Principal Investigator	Lead City University Toll-Gate, Off Oba Otudeko Avenue, Ibadan, Oyo State.
Date of Receipt of Valid Application:	17th January, 2023

NOTICE OF APPROVAL

This is to inform you that the research described in the submitted protocol, the consent forms, advertisements and other participant information materials have been reviewed and *given full approval by the Health Research Ethics Committee, National Hospital Abuja.*

This approval dates from **17th April, 2023** to **16th April, 2024.** If there is delay in starting the research, please inform the HREC National Hospital Abuja so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside of these dates. *All informed consent forms used in this study must carry the HREC assigned number and duration of HREC approval of the study.*

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the HREC. No changes are permitted in the research without prior approval by the HREC except in circumstances outlined in the Code. The HREC reserves the right to conduct compliance visit to your research site without previous notification.

7/4/23

Dr. Badejo Olawale Ayodele
(DCS/CMAC)
For: Chairman, HREC, National Hospital Abuja

GOVERNMENT OF RIVERS STATE OF NIGERIA

Neuropsychiatric Hospital,
Rumuigbo, Port Harcourt,
19-01-2023.

Obianuju Ibifuro Ojikah,
Lead City University,
Toll- Gate,
Off Oba Otundeko Avenue,
Ibadan, Oyo State.

Madam,

APPROVAL FOR RESEARCH
RE: OBIANUJU IBUFURO OJIKAH

I am directed to inform you that your request for a research work in our facility has been approved, knowing that it has to be carried out with utmost confidentiality. We shall accord you all necessary assistance.

Thanks for your cooperation.

Ibiomgbo Esther L.
Hospital Secretary.

NEURO PSYCHIATRIC HOSPITAL RUMUIGBO
19 JAN 2023
Sign.....

FEDERAL NEURO-PSYCHIATRIC HOSPITAL, YABA - LAGOS

8, Harvey Road, P.M.B. 2008 Yaba, Lagos, Nigeria. Tel: 0906 000 1907, 0815 517 0000
E-mail: enquiries@fnphyaba.gov.ng neuropsychiatrichospitalyaba@yahoo.com
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DIRECTOR OF ADMINISTRATION

FNPHY/HREC/2023/001/05/087 21st June, 2023

OBIANUJU IBIFURO OJIKAH

EFFECTS OF THE SL C6A4 GENE AND GONADOCORTICIODS IN SEROTONIN LEVELS AND INFLAMMATION IN SEVERITY OF DEPRESSION.

I am directed to inform you that your proposal has been reviewed and approved.

You are hereby granted permission to proceed with the research.

Conclusion: Approved

A copy of this final research study should be sent to the hospital library for record purpose.

DR.T.G.IJAROGBE
Chairperson
HREC

K.O.ISEGEN (Mrs)
Secretary
HREC

Note: that this approval expires if the research is not commenced within the next one year

Kindly equip yourself with enough copies of the official approval letter for future correspondence

14479

Lead City University (LCU)

Motto: Knowledge for Self-reliance
Lagos - Ibadan Expressway, Toll Gate Area, Ibadan, Oyo State, Nigeria
Email: lcu.hrec@lcu.edu.ng

NHREC

University Research Ethics Committee

PROJECT TITLE:	**EFFECTS OF SLC6A4 GENE AND GONADOCORTICIODS ON THE SEROTONIN LEVELS AND INFLAMMATION IN INDIVIDUALS SUFFERING FROM DEPRESSION**
PROJECT NUMBER:	**LCU-REC/24/010**
APPROVAL DATE:	**11/03/2024**
EXPIRY DATE:	**11/03/2025**

APPROVAL LETTER

The above-named proposal has been adequately reviewed: the protocol and safety guidelines satisfy the conditions of *LCU-REC* policies regarding experiments that use human subjects. Therefore, the study under its reviewed state is hereby approved by the LCU-Research Ethics Committee.

Prof. Olusola Ladokun

Name of LCU-REC Chairman

.....

Dr. Folahanmi Akinsolu

Name of LCU-REC Secretary

.....

This approval is given with the investigator's Declaration as stated below;

By signing below, I agree/certify that:

1. I have reviewed this protocol submission in its entirety and that I am fully cognizant of, and in agreement with all submitted statements.

2. I will conduct this research study in strict accordance with all submitted statements except where a change may be necessary to eliminate apparent immediate hazard to a given research subject.
   - I will notify the LCU-REC promptly of any change in research procedures necessitated in the interest of the safety of a given research subject.
   - I will request and obtain LCU-REC approval of any proposed modification to the research protocol or informed consent document(s) prior to implementing such modifications.

3. I will ensure that all co-investigator and other personnel assisting in the conduct of this research study have been provided a copy of the entire current version of the research protocol and are fully informed of the current (a) study procedures (including procedure modifications); (b) informed consent requirements and process; (c) potential risks associated with the study participation and the steps required to be taken to prevent or minimize these potential risks; (d) adverse events reporting requirements; (e) data and record-keeping; and (f) the current REC approval status of the research study.

4. I will respond promptly to all requests for information or materials solicited by the REC or REC Office.

5. I will submit the research study in a timely manner for the REC renewal approval.

6. I will not enroll any individual into this research study until such time I obtain his/her written informed consent, or if applicable, the written informed consent of his/her authorized representative (i.e unless the REC has granted a waiver of the requirement to obtain informed consent).

7. I will employ and oversee an informed consent process that ensures that potential research subjects understand fully the purpose of the research study, the nature of the research procedures they are being asked to undergo, the potential risks of these research procedures, and their rights as a research study volunteer.

8. I will ensure that the research subjects are kept fully informed of any new information that may affect their willingness to continue to participate in the research study.

9. I will maintain adequate, current, and accurate records of research data, outcomes, and adverse events to permit an ongoing assessment of the risks/benefits ratio of research study participation.

10. I am cognizant of, and will comply with, current federal regulations and REC requirements governing human subject research including adverse event reporting requirements.

11. I will make a reasonable effort to ensure that subjects who have suffered adverse event associated with research participation receive adequate care to correct or alleviate the consequences of the adverse event in the extent possible.

12. I will ensure that the conduct of this research study adheres to Good Clinical Practice guidelines.

Miss OJIKAH OBIANUJU .....

Principal Investigator's Name Principal Investigator's Signature and Date

Appendix 2: **Hamilton Depressive Rating Scale (HDRS)**

Hamilton Depression Rating Scale (HDRS)

Reference: **Hamilton M. A rating scale for depression. J Neurol Neurosurg Psychiatry 1960; 23:56–62**

Rating Clinician-rated

Administration time 20–30 minutes

Main purpose To assess severity of, and change in, depressive symptoms

Population Adults

Commentary

The HDRS (also known as the Ham-D) is the most widely used clinician-administered depression assessment scale. The original version contains 17 items ($HDRS_{17}$) pertaining to symptoms of depression experienced over the past week. Although the scale was designed for completion after an unstructured clinical interview, there are now semi-structured interview guides available. The HDRS was originally developed for hospital inpatients, thus the emphasis on melancholic and physical symptoms of depression. A later 21-item version ($HDRS_{21}$) included 4 items intended to subtype the depression, but which are sometimes, incorrectly, used to rate severity. A limitation of the HDRS is that atypical symptoms of depression (e.g., hypersomnia, hyperphagia) are not assessed (see SIGH-SAD, page 55).

Scoring

Method for scoring varies by version. For the $HDRS_{17}$, a score of 0–7 is generally accepted to be within the normal range (or in clinical remission), while a score of 20 or higher (indicating at least moderate severity) is usually required for entry into a clinical trial.

Versions

The scale has been translated into a number of languages including French, German, Italian, Thai, and Turkish. As well, there is an Interactive Voice Response version (IVR), a Seasonal Affective Disorder version (SIGH-SAD, see page 55), and a Structured Interview Version (HDS-SIV). Numerous versions with varying lengths include the HDRS17, HDRS21, HDRS29, HDRS8, HDRS6, HDRS24, and HDRS7 (see page 30).

Additional references

Hamilton M. Development of a rating scale for primary depressive illness. Br J Soc Clin Psychol 1967; 6(4):278–96.

Williams JB. A structured interview guide for the Hamilton Depression Rating Scale. Arch Gen Psychiatry 1988; 45(8):742–7.

Address for correspondence

The HDRS is in the public domain.

Hamilton Depression Rating Scale (HDRS)

PLEASE COMPLETE THE SCALE BASED ON A STRUCTURED INTERVIEW

Instructions: for each item select the one "cue" which best characterizes the patient. Be sure to record the answers in the appropriate spaces (positions 0 through 4).

1 DEPRESSED MOOD *(sadness, hopeless, helpless, worthless)*

0 |__| Absent.
1 |__| These feeling states indicated only on questioning.
2 |__| These feeling states spontaneously reported verbally.
3 |__| Communicates feeling states non-verbally, i.e. through facial expression, posture, voice and tendency to weep.
4 |__| Patient reports virtually only these feeling states in his/her spontaneous verbal and non-verbal communication.

2 FEELINGS OF GUILT

0 |__| Absent.
1 |__| Self reproach, feels he/she has let people down.
2 |__| Ideas of guilt or rumination over past errors or sinful deeds.
3 |__| Present illness is a punishment. Delusions of guilt.
4 |__| Hears accusatory or denunciatory voices and/or experiences threatening visual hallucinations.

3 SUICIDE
0 |__| Absent.
1 |__| Feels life is not worth living.
2 |__| Wishes he/she were dead or any thoughts of possible death to self.
3 |__| Ideas or gestures of suicide.
4 |__| Attempts at suicide (any serious attempt rate 4).

4 INSOMNIA: EARLY IN THE NIGHT
0 |__| No difficulty falling asleep.
1 |__| Complains of occasional difficulty falling asleep, i.e. more than ½ hour.
2 |__| Complains of nightly difficulty falling asleep.

5 INSOMNIA: MIDDLE OF THE NIGHT
0 |__| No difficulty.
1 |__| Patient complains of being restless and disturbed during the night.
2 |__| Waking during the night – any getting out of bed rates 2 (except for purposes of voiding).

6 INSOMNIA: EARLY HOURS OF THE MORNING
0 |__| No difficulty.
1 |__| Waking in early hours of the morning but goes back to sleep.
2 |__| Unable to fall asleep again if he/she gets out of bed.

7 WORK AND ACTIVITIES
0 |__| No difficulty.
1 |__| Thoughts and feelings of incapacity, fatigue or weakness related to activities, work or hobbies.
2 |__| Loss of interest in activity, hobbies or work – either directly reported by the patient or indirect in listlessness, indecision and vacillation (feels he/she has to push self to work or activities).
3 |__| Decrease in actual time spent in activities or decrease in productivity. Rate 3 if the patient does not spend at least three hours a day in activities (job or hobbies) excluding routine chores.
4 |__| Stopped working because of present illness. Rate 4 if patient engages in no activities except routine chores, or if patient fails to perform routine chores unassisted.

8 RETARDATION (slowness of thought and speech, impaired ability to concentrate, decreased motor activity)
0 |__| Normal speech and thought.
1 |__| Slight retardation during the interview.
2 |__| Obvious retardation during the interview.
3 |__| Interview difficult.
4 |__| Complete stupor.

9 AGITATION
0 |__| None.
1 |__| Fidgetiness.
2 |__| Playing with hands, hair, etc.
3 |__| Moving about, can't sit still.
4 |__| Hand wringing, nail biting, hair-pulling, biting of lips.

10 ANXIETY PSYCHIC
0 |__| No difficulty.
1 |__| Subjective tension and irritability.
2 |__| Worrying about minor matters.
3 |__| Apprehensive attitude apparent in face or speech.
4 |__| Fears expressed without questioning.

11 ANXIETY SOMATIC (physiological concomitants of anxiety) such as:
gastro-intestinal – dry mouth, wind, indigestion, diarrhea, cramps, belching
cardio-vascular – palpitations, headaches
respiratory – hyperventilation, sighing
urinary frequency
sweating
0 |__| Absent.
1 |__| Mild.
2 |__| Moderate.
3 |__| Severe.
4 |__| Incapacitating.

12 SOMATIC SYMPTOMS GASTRO-INTESTINAL
0 |__| None.
1 |__| Loss of appetite but eating without staff encouragement. Heavy feelings in abdomen.
2 |__| Difficulty eating without staff urging. Requests or requires laxatives or medication for bowels or medication for gastro-intestinal symptoms.

13 GENERAL SOMATIC SYMPTOMS
0 |__| None.
1 |__| Heaviness in limbs, back or head. Backaches, headaches, muscle aches. Loss of energy and fatigability.
2 |__| Any clear-cut symptom rates 2.

14 GENITAL SYMPTOMS (symptoms such as loss of libido, menstrual disturbances)
0 |__| Absent.
1 |__| Mild.
2 |__| Severe.

15 HYPOCHONDRIASIS
0 |__| Not present.
1 |__| Self-absorption (bodily).
2 |__| Preoccupation with health.
3 |__| Frequent complaints, requests for help, etc.
4 |__| Hypochondriacal delusions.

16 LOSS OF WEIGHT ***(RATE EITHER a OR b)***

a) According to the patient:	**b) According to weekly measurements:**
0 |__| No weight loss.	0 |__| Less than 1 lb weight loss in week.
1 |__| Probable weight loss associated with present illness.	1 |__| Greater than 1 lb weight loss in week.
2 |__| Definite (according to patient) weight loss.	2 |__| Greater than 2 lb weight loss in week.
3 |__| Not assessed.	3 |__| Not assessed.

17 INSIGHT
0 |__| Acknowledges being depressed and ill.
1 |__| Acknowledges illness but attributes cause to bad food, climate, overwork, virus, need for rest, etc.
2 |__| Denies being ill at all.

Total score: |__|__|

Appendix 3: **Socio-economic Questionn**

Socio-Economic Questionnaire.

Effects of the SLC6A4 Gene and Gonadocorticiods in Serotonin Levels and Inflammation in Severity of Depression.

Please note that all information provided within this document will be kept confidential before, during and after the duration of this research work.

Date:_____

This questionnaire aims at providing personal, health and economic information on the participants of this study. This will enable researchers in this study to gain insight on external and environmental factors that may play a role or have significant associations with the population present within this study.

Participants are encouraged to be honest with the information provided in this form. This will aid informed projections to be made on the subject matter.

Personal Information:

Title:_____

Name:_____

Age:_____

Contact Information(phone number or email address):

_____

Sex: ☐**Male** ☐**Female**

Marital Status: ☐**Single** ☐**Married** ☐**Divorced**

How many children do you have ? please state the number of children if yes

_____

Are you a bread winner of you nuclear / extended family?
☐ **Yes, I am.** ☐ **No, I am not.**

Are you currently experiencing any marital/family issues?
☐ **Yes, I am.** ☐ **No, I'm not.**

Have these issues been been long standing, recent or re-occurring? please indicate by underlining your answer.

Qualifications:

Level of Education: ☐Primary School ☐ Secondary School ☐ University ☐ Post-Graduate

Qualifications:_____

Current Occupation:_____

- Earning Status : ☐ Low Income Earner ☐ Minimum Wage Earner ☐ High Income Earner

- Are there any activities at your work place that may cause you a significant amount of distress ?

_____

_____

_____

Is your income sufficient for yourself and dependants?
☐Yes, it is. ☐ No it is not

- Do you enjoy your job? ☐ Yes, I do. ☐No, I do not.

Dietary Information

What are staple foods you eat? Please list below

_____

_____

Do you consume diary foods (examples: milk, cheese, yoghurt)?

☐ Not at all ☐ Rarely ☐ Often ☐ In excess

Do you smoke cigarettes ? ☐Yes, I do. ☐No, I don't .

If yes, how often?

☐ Not at all ☐ Rarely ☐ Often ☐ In excess

Do you drink alcohol ? ☐Yes, I do. ☐No, I don't

If yes, how often?

☐ Not at all ☐ Rarely ☐ Often ☐ In excess

Do you exercise ? ☐Yes, I do. ☐No, I don't

If yes, how often do you exercise in a week and are you consistent?

_____

Are you on supplements? ☐Yes, i am. ☐ No, i am not

If yes, please list supplements being taken (examples: iron supplements, calcium supplements multivitamins etc.):

_____

_____

Do you have any significant health issues (example: high blood pressure, diabetes etc) ?
☐Yes, I do. ☐No, I don't

If yes please state below:

_____

Have you ever been diagnosed wit hormonal imbalance prior to this study?

☐Yes, I have. ☐No, I haven't.

Do you have any genetic disorder? If yes please state below

_____

Have you been diagnosed with depression prior to this interaction?

☐Yes, I have. ☐No, I haven't

If yes, what type of medication were you placed on?

_____

Did the medication provide you with any notable improvement?

☐Yes, it did. ☐No, i did not.

THANK YOU FOR PARTAKING IN THIS RESEARCH STUDY. YOUR PARTICIPATION IS HIGHLY APPRECIATED.

Appendix 4: **Consent Form**

National Hospital Abuja.

Consent to Act as a Research Participant

Effects of the SLC6A4 Gene and Gonadocorticiods in Serotonin Levels and Inflammation in Severity of Depression in Nigeria.

Introduction

Miss Obianuju Ojikah, a staff at Baze University, FCT, Abuja and a PhD candidate at Lead City Univeristy. Ibadan, Oyo State. The title of this study "***Effects of the SLC6A4 Gene and Gonadocorticiods in Serotonin Levels and Inflammation in Severity of Depressionin Nigeria***". She is asking for your consent to participate. If you choose not to participate, there will be no penalties as you will receive the standard care available in this facility.

This research is privately sponsored because despite available pharmaco-therapeutic options, 30–60% of patients with MDD are not responsive , the rate of remission of the disease is often < 50%. Asides the expected and many side effects, interactions between anti-depressants and other drugs usually are detrimental to the hosts. There is no compelling evidence that current treatments are capable of disease modification in MDD patients. This research aims at additional treatment modalities with higher specificity to genetic backgrounds to be developed because the effectiveness of SSRIs in treating depression with a response rate that is still still moderate (about 50%).

This document will provide you with information about the research. In summary,

- Research is voluntary - whether you join is your decision. You can discuss your decision with others (such as family, friends or another physician).
- You can say yes but change your mind later.
- If you say no, we will not hold your decision against you. You will receive routine care.
- Your decision will not affect your health care or any other benefits you may be entitled to.
- You can say no even if the person inviting you is part of your healthcare team.
- Please ask questions or mention concerns before, during or after the research.
- Participation in the study will be beneficial to you directly. Much more, we hope it will generate new knowledge to improve the treatment programme and thus may help others.

Purpose of Study: The purpose of this study is to determine the relationship between SLC6A4 gene its effects on hormonal levels in the etiology of depression in the both men and women.

Main procedure for a typical participant

This study will involve individuals (both men and women) living with depression in Nigeria.

Prior to Testing

If you agree to participate, you will receive a higher standard of care, with additional tests and possibly regimen change to ensure remission does not occur. First, you will undergo SCL6A4 gene testing, hormonal levels (serotonin and gonadocorticoids)

"SCL6A4" gene testing means taking genetic information from your blood and doing a test to see what type of gene (long or short). Individuals will be categorised into long or short genotypes. Ussing the blood samples, serotonin and gonatocorticoid levels (progesterone, testosterone and estrogen). The test report will provided to your doctor to enable them decide how well the course of your treatment will proceed and if there is a need for change of treatment.

The report of the gene testing will be used to improve your treatment to ensure all drugs are working very well and hopefully, your viral load will become too low to detect before you are due to deliver your baby. If you are just starting treatment for the first time, you will be put on the standard regimen until the gene testing report is out. The testing report will subsequently be used to personalize your treatment to ensure you are receiving the most effective drugs that are suitable for you.

Expected Benefits of this Research: This study will aid in elucidating the pathophysiology of depression, therefore being able to detect those who are at a predisposition in Nigerians and also help in treatment regimens reducing the chances of remission. The individuals participating in this study will receive additional laboratory testing that will help their doctors take better care of them with a genetic background and other serological information that will be very crucial to patients' wellness.

Risks: The risks associated with this study include a few additional visits to the clinic, the slight discomfort you will feel when your blood will be collected and the risk of finding out that your treatment may not be working very well, necessitating a change of drug(s). Additional, detailed information about this research is provided below. Please feel free to ask questions before signing this consent form.

Why have you been asked to participate, how you were selected, and what is the approximate number of participants in the study?

You have been asked to participate in this study because you are of Nigerian decent, an adult (above 18 years old) and are currently suffering from depression or are being recruited as part of the control population of this study but must still meet the first two requirements. You were selected as a result of your enrollment at the clinic in the institute. There will be approximately 210 participants in this study.

What will happen to you in this study and which procedures are standard of care and which are experimental?

In addition to the information at the beginning of this form, here are some additional details about what will happen to you if you agree to be in this study.

To carry out your SCL6A4 gene determination and hormonal levels testing, about 10 mL of blood will be collected from patients via venous puncture using a needle.

How much time will each study procedure take and how long will the study last?

For you, the study is intended to cover just the collection of blood samples. This will be done within a few minutes by a trained professional and fill two questionnaire forms.

What risks are associated with this study?
Participation in this study has minimal risk involved but may involve some added discomforts. In addition to the risks described at the beginning of this form, the most expected discomfort is the mild pain from needle puncture during blood collection. If there are unknown risks currently unforeseen, you will be informed of any significant new findings/change and be given the opportunity to continue or to withdraw from the study.

What benefits can be reasonably expected? A direct benefit to you will be the customization of treatment and a higher standard of care due to the additional testing, which the study will pay for. The reports from these laboratory tests will help your doctor take better care of you during this study.

What happens if you change your mind about participating? If you decide that you no longer wish to continue in this study, you will be requested to notify the principal investigator, Obianuju Ojikah or the people authorizing the study to report any event you do not like. During this study, you will be informed of any new information important in helping you decide to continue or to withdraw from the study.

Can you be withdrawn from the study without your consent?
You may be withdrawn from the study if you it is discovered that you have had recent apparent loss therefore triggering the Depressive symptoms or head trauma.

Will you be compensated for participating in this study? There are no monetary benefits or compensation beyond the free provision of additional testing. These testing are expensive and that is why the government cannot afford to offer it routinely. However, if you choose to participate, the study will provide these tests free of charge to you. The test reports will help your doctor take better care of you.

Are there any costs associated with participating in this study?
No.

What about your confidentiality? Extensive precautions will be taken to keep your personal information and medical records safe. Access to your records will only be as needed to serve you better. For example, your doctor will have access to your laboratory tests to take care of you. However, the blood samples will be given
a code while being tested in the laboratory to protect your personal details. Only the Principal investigator will have access to who each code belongs to. This will be used when reporting back the test results to your doctor. The study documents will be protected, using a locked cabinet for hard-copy documents and an encrypted computer for electronic documents. Your medical and research records will be kept confidential
to the extent allowed by law.

Personal details that can be used to identify you will be removed from your blood and swab samples collected as part of the research. Your information, documents, or bio-specimens will not be disclosed to anyone else who is not connected with the research *unless* there is a federal, state, or local law that requires disclosure (such as to report child abuse, elder abuse, intent to hurt self or others, or communicable diseases), you have consented to the disclosure, including for your medical treatment; or it is used for the scientific research, as allowed by federal regulations protecting research subjects.

You should also understand that we do not prohibit you from voluntarily releasing information about yourself or your involvement in this research. If you want your research information released to an insurer, medical care provider, or any other person not connected with the research, you must provide consent to allow the research to release it.

Will you receive any results from participating in this study? Clinically relevant results will be disclosed to you by the clinical team taking care of you after the study provided your additional test results.

Who can you call if you have questions? If you should have any questions about this study, please feel free to contact the principal investigator: **Miss OBIANUJU OJIKAH** (obianujuojikah@gmail.com; 08173115342 or 07054647125) prject supervisor: **Dr C.K. ONWUAMAH** (chikaonwuamah@yahoo.com; 09098058007).

STATEMENT BY PERSON AGREEING TO PARTICIPATE IN THIS STUDY

Participation in this study is voluntary and there are no penalties for not participating. You will receive a copy of this consent document to keep.

Do you agree to participate?

☐ Yes, you will be in this research study. ☐ No, you don't want to do this.

I have read and understood this informed consent document and the information has been explained to me verbally. All my questions have been answered, and I freely and voluntarily choose to participate.

Participant's Name	**Signature**	**Date**
Independent Witness (Name)	**Signature**	**Date**
Research Team Member (Name)	**Signature**	**Date**

STATEMENT BY PERSON AGREEING TO PARTICIPATE IN THIS STUDY (Duplicate)

Participation in this study is voluntary and there are no penalties for not participating. You will receive a copy of this consent document to keep.

Do you agree to participate?

☐ Yes, you will be in this research study. ☐ No, you don't want to do this.

I have read and understood this informed consent document and the information has been explained to me verbally. All my questions have been answered, and I freely and voluntarily choose to participate.

Participant's Name	**Signature**	**Date**
Independent Witness (Name)	**Signature**	**Date**
Research Team Member (Name)	**Signature**	**Date**

Appendix 5: **Confidentiality Agreement**

CONFIDENTIALITY AGREEMENT

This Confidentiality Agreement ("Agreement") is executed effective_____.

BETWEEN: _____, her, parents, successors, heirs assignee's and other legal representatives ("Researcher") of the Research Study "Effects of SCL6A4 gene and Gonadocorticiods on the Serotonin Levels and Inflammation in Individuals Experiencing Depression." or
"Role Of The Scl6A4 Gene (5HTTLPR And rs6354 & rs8076005), Serotonin Levels, Gonadocorticoids And Inflamation In Severity Of Depression In Three Geo-Political Zones In Nigeria." ("Research"),

_____
(ADDRESS)

AND: _____ his, spouse, heirs, subsidiaries, parents, successors, assignnee's or other legal representative ("Analyst"),

_____
(ADDRESS)

As consideration for the establishment and/or continuation of their employment relationship and sharing of Confidential Material. The parties agree as follows:

1. **Length of Agreement.** This Agreement begins retroactively to the beginning of Analyst's relationship with Researcher and remains in effect at all times during any consulting, partnering, or other business relationship between the parties and for the periods of time specified thereafter as set forth below. This Agreement does not create any form of continued business relationship other than as set forth in a separate written agreement signed and dated by all parties.

2. **Confidentiality.** Analyst hereby acknowledges that Researcher has made, or may make, available to Analyst certain data, supply sources, techniques, computerized data, maps, methods, design information, technical information, benchmarks, performance standards and other confidential and/or Proprietary Information of, or licensed to, the Researcher, including without limitation, inventions, patents, and copyrighted materials (collectively, the "Confidential Material"). Analyst acknowledges that this information has independent economic value, actual or potential, that is not generally known to the public or to others who could obtain economic value from their disclosure or use, and that this information is subject to a reasonable effort by the Researcher to maintain its secrecy and confidentiality. Except as essential to Analyst's obligation under this Agreement, Analyst shall not make any disclosure of this

Agreement, the terms of this Agreement, or any of the Confidential Material. Analyst shall not remove Confidential Material or proprietary property or documents without written authorization. Immediately upon request from Researcher, Analyst shall return to Researcher all Confidential Material or proprietary property or documents in his possesion.

3. **Proprietary Information.** For the purpose of this Agreement, "Proprietary Information" shall include, but not limited to any information, observation, data, written material, record, document, drawing, photograph, layout, computer program, software, multimedia, firmware, invention, discovery, improvement, development, tool, design, work of authorship, Title, whole or part, system, promotional idea, publication, article, practice, journal, process, test, concept, formula, method, field information, technique, product and/or research related to the actual or anticipated research development, products, organization, advertising, or finances of Researcher, its affiliates or related entities.

All right, title, and interest of every kind and nature whatsoever in and to the Proprietary Information made, written, discussed, developed, secured, obtained or learned by Analyst during the term of the relationship with the Researcher or the _____[time] period immediately following relationship (in perpetuality), shall be the sole and exclusive property of Researcher for any purpose or use whatsoever, and shall be disclosed promptly by Analyst to Researcher. The covenants set forth in the preceding sentence shall apply regardless of whether any Propriety Information is made, written, discussed, developed, secured, obtained or learned (a) solely or jointly with others, (b) during the usual hours of work or otherwise, (c) at the request and upon the suggestion of Researcher or otherwise, (d) with Researcher's materials, tools, instruments, or (e) on Researcher's premises or otherwise.

Analyst shall promptly and fully disclose to Researcher, in confidence all Proprietary Information that Analyst creates, conceives or reduces to practice in writing either alone or with others during the term or pertainig to this study.

Nothing contained in this Agreement shall be construed to preclude Researcher from exercising all of her rights and privileges as sole and exclusive Researcher of all of the Proprietary Information owned by or assigned to Researcher under this Agreement.

Researcher, in exercising such rights and privileges with respect to any particular item of Proprietary Information, deciding/not deciding to file any patent application or any copyright registration on such Proprietary Information, deciding to maintain such Proprietary Information as secret and confidential, or deciding to abandon such Propriety Information, or dedicate it to the public. Analyst shall have no authority to exercise any rights or privileges with respect to the Proprietary Information owned by or assigned to Researcher under this Agreement.

4. **Analyst's Obligations.** Analyst agrees that the Confidential Information is to be considered confidential and proprietary to Researcher and Analyst shall hold the same in confidence, shall not use the Confidential Information other than for the purposes of its business with Researcher, and shall disclose it only to its officers, directors, or employees with a specific need to know. Analyst will not disclose, publish or

otherwise reveal any of the Confidential Information received from Researcher to any other party whatsoever except with the specific prior written authorization of Researcher.

Confidential Information furnished in tangible form shall not be duplicated by Analyst except for purposes of this Agreement. Upon the request of Researcher, Analyst shall return all Confidential Information received in written or tangible form, including copies(hard/soft copies), or reproductions or other media containing such Confidential Information, within **SEVEN (7)** working days of such request. At Analyst's option, any documents or other media developed by the Analyst containing Confidential Information should be destroyed by Analyst. Analyst shall provide a written certificate to Researcher regarding destruction with evidence (photo/video) within **SEVEN (7)** working days thereafter.

5. **Term.** The obligations of Analyst herein shall be effective [NON-DISCLOSURE PERIOD] from the date Researcher last discloses any Confidential Information to Analyst pursuant to this Agreement. Further, the obligation not to disclose shall not be affected by bankruptcy, receivership, assignment, attachment or seizure procedures, whether initiated by or against Analyst, nor by the rejection of any agreement between Researcher and Analyst, by a trustee of Analyst in bankruptcy, or by the Analyst as a debtor-in-possession or the equivalent of any of the foregoing under local law.

6. **Confidentiality.** Analyst and its Representatives shall not disclose any of the Confidential Information in any manner whatsoever, except as provided in Articles 7 of this Agreement, and shall hold and maintain the Confidential Information in strictest confidence. Analyst hereby agrees to indemnify Researcher against any and all losses, damages, claims, expenses, and attorneys' fees incurred or suffered by Researcher as a result of a breach of this Agreement by Analyst or its Representatives.

7. Permitted Disclosures. Analyst may disclose Researcher's Confidential Information to Analyst's responsible Representatives with a bona fide need to know such Confidential Information, but only to the extent necessary to evaluate or carry out a proposed transaction or relationship with Researcher and only if such employees are advised of the confidential nature of such Confidential Information and the terms of this Agreement and are bound by a written agreement or by a legally enforceable code of professional responsibility to protect the confidentiality of such Confidential Information. and Research.

7. **Works for Hire.** Analyst acknowledges that all works of authorship performed for Researcher are subject to Researcher's direction and control and that such works constitute a work for hire.

All Propriety Information developed, created, invented, devised, conceived or discovered by Analyst that is subject to copyright are explicitly considered by Analyst and Researcher to be "works made for hire" and the property of Researcher.

8. **Assignment.** Researcher shall own as its sole and exclusive property, and Analyst agrees to assign, transfer, and convey and or its authorized nominees all of his or her right, title and interest in and to any and all said "ideas" that related generally to Researcher's study, including but not limited to any inventions, processes,

improvements, ideas, trademarks, copyrights, formulas, manufacturing technology, developments, writings, discoveries, that Analyst may make, conceive, or reduce to practice, whether solely or jointly with others, copyrightable, patentable or unpatentable, from the date of this Agreement or the date of first employment with Researcher if earlier, until the termination of Analyst's employment and even after.

9. **No Ownership.** Neither Analyst nor any of their agents or principals shall become or be deemed a Researcher, partner, joint venture or agent of or with Researcher as regards to this Research Study by reason of this Agreement or his/her relationship with Researcher unless set forth in a separate written agreement signed and dated by the parties. Neither Researcher nor Analyst nor any agent, Analyst, officer or independent contractor of or retained by Analyst shall have any authority to bind the other in any respect unless set forth in a separate written agreement signed and dated by the Resarcher.

10. **Injunctive Relief.** Analyst hereby acknowledges (1) the unique nature of the protections and provisions set forth in this Agreement, (2) that Researcher will suffer irreparable harm if Analyst breaches any of said protections or provisions, and (3) that monetary damages will be inadequate to compensate Researcher for such breach. Therefore, if Analyst breaches any of such provisions, then Researcher shall be entitled to injunctive relief, in addition to any other remedies at law or equity, to enforce such provisions.

11. **Continuing Effects.** Analyst's obligations regarding trade secrets and confidential information shall continue in effect beyond the period of the relationship as stated above in article 3 paragraph 2, and said obligation shall be binding upon Analyst's spouse, affiliates, assignees, heirs, executors, administrators, or other legal representatives.

12. **Non-Filing.** Analyst specifically agrees that Researcher's rights granted hereunder shall include the right not to file for copyrights or domestic or foreign patents when such is considered by Researcher in her sole discretion appropriate for the Study objectives of Researcher.

13. **Notice to Analyst.** This Agreement does not apply to any invention for which no equipment, supplies, facility, or trade secret information of Researcher was used and that was developed entirely on Analyst's own time and:

I. That does not relate (1) to Researcher's Study or (2) to the actual or anticipated research or development work of Researcher; or

II. That does not result from any work performed by Analyst or Researcher. The burden of proof is on the Analyst with respect to the exceptions of this Paragraph.

14. **Drafting Ambiguities.** Each party to this Agreement has reviewed and had the opportunity to revise this Agreement. Each party to this Agreement has had the opportunity to have legal counsel review and revise this Agreement. The rule of construction that any ambiguities are to be resolved against the drafting party shall not be employed in the interpretation of this Agreement or of any amendments or exhibits to this Agreement.

15. **Receipt of Copy.** Analyst hereby acknowledges that he/she/it has received a signed copy of this Agreement.

BY: _____	BY: _____
Title	Title
_____	_____
Analyst	Researcher
_____	_____
Date	Date

Appendix 6: **Gene Assay Information**

Assay Information

Custom TaqMan® Copy Number Assays

Sales Order Number:	7428615	**Customer Name:**	PETER AND JANE LIMITED
Rack or Array ID:	8266295_2	**Contact Name:**	chandrakant kulkarni
Rack or Array Type:	96-position tube rack v1	**Ship Date:**	19-FEB-2024
Vial Type:	Matrix Tube	**XML Template Version:**	2.0

Link to Annotation Section
Link to Design Details Section
Link to Assay Information Field Descriptions HTML Page

Contents

Tube/Well Position	Assay ID	Product Type	Part Number	Expiration Date	Vial ID	Lot Number	Annotation Sources
A01	HTTLPR_CDT2AAY	Custom TaqMan® Copy Number Assays, SM	4400294	11-FEB-2029	0447943166	P240213-009A01	NA

Annotation Information

Design Details (Back to Contents)

Assay ID	Assay Mix Concentration	Reporter 1 Dye	Reporter 1 Concentration (µM)	Reporter 1 Quencher	Forward Primer Concentration (µM)	Reverse Primer Concentration (µM)	Forward Primer Sequence	Reverse Primer Sequence	Reporter 1 Sequence	Amplicon Size
HTTLPR_CDT2AAY	20x	FAM	5	NFQ	18	18	GAAACCCAATTGGCAGAAACTC	GAAGATCTGAGCGGCTGCAT	ATCCACACCCCTGTCT	NA

NA = Not applicable or not available.

TaqMan® SNP Genotyping Assays

Sales Order Number:	7428615	**Customer Name:**	PETER AND JANE LIMITED
Rack or Array ID:	8266295_1	**Contact Name:**	chandrakant kulkarni
Rack or Array Type:	96-position tube rack v1	**Ship Date:**	16-FEB-2024
Vial Type:	Matrix Tube	**XML Template Version:**	2.0

Link to Annotation Section
Link to Design Details Section
Link to Assay Information Field Descriptions HTML Page

Contents

Tube/Well Position	Assay ID	Product Type	Part Number	Expiration Date	Vial ID	Lot Number	Annotation Sources
A01	C___1841706_20	TaqMan® SNP Genotyping Assays, Human, SM	4351379	11-FEB-2029	0447942376	P240213-010C10	NCBI, SNP

Annotation Information

NCBI (Back to Contents)

Assay ID	Gene Symbol	Gene Name	Entrez Gene ID	Transcript Accession	Species	Cytogenetic Band	NCBI Assembly Build Number	Chromosome	Loca
C___1841706_20	SLC6A4	solute carrier family 6 member 4	6532	NM_001045.5	Homo sapiens	17q11.2	GRCh38	17	

SNP (Back to Contents)

Assay ID	NCBI SNP Reference	Celera SNP ID	SNP Type	Amino Acid Change	AB Minor Allele Frequency - African-American	AB Minor Allele Frequency - Caucasian	AB Minor Allele Frequency - Chinese	AB Minor Allele Frequency - Japanese	HapMap Minor Allele Freq - YRI	A
C___1841706_20	rs6354	hCV1841706	UTR 5	NA	NA	NA	NA	NA	0.32	

Design Details (Back to Contents)

Assay ID	Assay Mix Concentration	Reporter 1 Dye	Reporter 1 Concentration (µM)	Reporter 1 Quencher	Reporter 2 Dye	Reporter 2 Concentration (µM)	Reporter 2 Quencher	Forward Primer Concentration (µM)	Reverse Primer Concentration (µM)	Conte
C___1841706_20	40x	VIC	8	NFQ	FAM	8	NFQ	36	36	GGCTAAGCCCCTTGTTATTCTGCAA

NA = Not applicable or not available.

Assay Information

TaqMan® SNP Genotyping Assays

Sales Order Number:	7428615	**Customer Name:**	PETER AND JANE LIMITED
Rack or Array ID:	8266295_3	**Contact Name:**	chandrakant kulkarni
Rack or Array Type:	96-position tube rack v1	**Ship Date:**	20-FEB-2024
Vial Type:	Matrix Tube	**XML Template Version:**	2.0

Link to Annotation Section
Link to Design Details Section
Link to Assay Information Field Descriptions HTML Page

Contents

Tube/Well Position	Assay ID	Product Type	Part Number	Expiration Date	Vial ID	Lot Number	Annotation Sources
A01	C__28964485_10	TaqMan® SNP Genotyping Assays, Human, SM	4351379	13-FEB-2029	0447996197	P240215-008A06	NCBI, SNP

Annotation Information

NCBI (Back to Contents)

Assay ID	Gene Symbol	Gene Name	Entrez Gene ID	Transcript Accession	Species	Cytogenetic Band	NCBI Assembly Build Number	Chromosome	Location on NCBI Genome Assembly	Location on Transcript or Gene
C__28964485_10	SLC6A4	solute carrier family 6 member 4	6532	NM_001045.5	Homo sapiens	17q11.2	GRCh38	17	30220192	

SNP (Back to Contents)

Assay ID	NCBI SNP Reference	Celera SNP ID	SNP Type	Amino Acid Change	AB Minor Allele Frequency - African-American	AB Minor Allele Frequency - Caucasian	AB Minor Allele Frequency - Chinese	AB Minor Allele Frequency - Japanese	HapMap Minor Allele Freq - YRI	HapMap Minor Allele Freq - CEU	HapMap Minor Allele Freq - CHB	HapMap Minor Allele Freq - JPT
C__28964485_10	rs8076005	hCV28964485	Intron	NA	NA	NA	NA	NA	0.29	0.18	0.11	0.12

Design Details (Back to Contents)

Assay ID	Assay Mix Concentration	Reporter 1 Dye	Reporter 1 Concentration (µM)	Reporter 1 Quencher	Reporter 2 Dye	Reporter 2 Concentration (µM)	Reporter 2 Quencher	Forward Primer Concentration (µM)	Reverse Primer Concentration (µM)	Context Sequence	Amplicon Size
C__28964485_10	40x	VIC	8	NFQ	FAM	8	NFQ	36	36	CAGGCCACTCTGTGTTAGGTCACGG[A/G]AGAAGTGAGGGTGACACAAATGGAT	NA

NA = Not applicable or not available.

Appendix 7: **Gene Assay Information**

Lcu Library

Obianuju_Ibifuro_Ojikah LCU Library

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Quick Submit

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Appendix 8: Collaborators

Institutional Collaborators.

- Federal Ministry of Health, F.C.T, Abuja
- National Hospital, Garki, F.C.T, Abuja
- Yaba Psychiatric Hospital, Yaba, Lagos State.
- Neuropsychiatric Hospital, Rumigbo, Port Harchourt, Rivers State.
- Lead City University, Ibadan, Oyo State.
- National Institute of Medical Research (NIMR), Yaba, Lagos.
- Baze University, Airport Road, FCT, Abuja.

Professional Collaborators and Study Team

1. Dr. Alex Kasembeli, Ph.D.

Clinical Research, Public Health/Administration and Laboratory Quality Management Systems. Thermofisher Scientific, Kenya.

2. Dr. Victor Chiemeka OJIAKU

Consultant Psychiatrist/Medical Director

Neuropsychiatric Hospital, Port Harcourt, Rivers State.

Specialization: Psychiatrist

3. Mrs Clinton Vivien Tari

Director Medical laboratory sciences

Head of Department Medical Laboratory Services in Neuropsychiatry Hospital

Rumuigbo, Port Harcourt, Rivers State.

Specialization: Medical Microbiology/Parasitology.

4. Dr Emmanuel Akhaumere Head of Department

Deputy Director / Head of Department Medical Laboratory Services, National Hospital, Garki, FCT. Abuja.

Specialization: Clinical Chemistry.

5. Mrs. Momoh Abidemi Esther

Project Manager, Population Genomics and Cancer, NIMR, Yaba, Lagos.

Specialization: Molecular Biologist.

6. Mrs. Agharanya Charity Chizobam

Portfolio Chief Medical Laboratory scientist, Chemical Pathology Department., National Hospital, Garki, FCT. Abuja.

Specialization: Immunology.

7. Dr Oluwatemitope Ajayi MB; BS MWACP

Senior Registrar Psychiatry, Federal Neuropsychiatric Hospital, Yaba, Lagos.

Specialization: Psychiatrist.

8. Ojo Temitope MBChB, MWACP, MRCPsych

Senior Registrar Psychiatry, Federal Neuropsychiatric Hospital, Yaba, Lagos.

Specialization: Psychiatrist.

9. Mr. Morah Ifeanyi.C.

Principal Medical Laboratory Scientist, Chemical Pathology Department.

Unit: Immunoassay/Tumor Markers.

Assistant Coordinator, intern medical laboratory scientist, chemical pathology department.

Specialization: Immunology.

10. Helen Jack-Ojikah BL LLB

Legal Aid Council of Nigeria.

Legal Consultant/ Adviser.

Bio-data

A. Personal Data

1. Full Name: Obianuju Ibifuro OJIKAH

5th Avenue, Gwarinpa Estate, F.C.T Abuja.

obianujuojikah@gmail.com

08173115342

2. Date of Birth: 24th March, 1999.

3. Nationality: Nigerian.

4. State of Origin: Anambra State.

5. Local Government Area: Nnewi-South.

6. Name and Address of Next of Kin: Helen Jack-Ojikah (Mother)

5th Avenue, Gwarinpa Estate, F.C.T Abuja.

B. Educational Background:

Educational Institutions Attended with Dates and Qualifications:

Institute Attended	**Dates**	**Qualification**
1. Day Spring College F.C.T Abuja	2010-2015	WAEC
2. Baze University F.C.T Abuja	2015-2018	B.Sc.
3. Lead City University, Ibadan, Oyo State	2018-2020	M.Sc.
4. Lead City University, Ibadan, Oyo State	2022-2025	Ph.D (in view)

C. **Working Experience with Dates:**

Working Experience	**Dates:**
1. F.C.T Health Insurance Scheme, F.C.T Abuja (NYSC)	2020-2021
2. Baze University F.C.T Abuja	2021- Till Date

D. Publications

1. Effects of Vitamin E and Selenium Yeast on Cognitive Performance of Pups Whose Dams were Subjected to Prenatal Noise Stress, Science Direct, 2023.

2. Flavonoids from Hibiscus sabdariffa Reduce Serum Aldosterone Level of Hypertensive Male Wistar Rats, International Journal of Medicine and Health Development, 2024.

3. Haptoglobin Phenotype, Serum Iron Levels and Severity in Multi-Drug Resistant Mycobacterium Tuberculosis Patients in Ibadan, Lead City Faculty of Applied Science Conference, 2023.

4. Nutritional Values and Effects of Selected Solvents on the Phytochemical Properties of Moringa Oleifera Leaves Obtained from Sokoto, Nigeria, 2024 International Conference on Science, Engineering and Business for Driving Sustainable Development Goals (SEB4SDG) IEEE, 2024.

…… ……

Signature Date

The University Compliance Certification

This is to certify that the thesis by Obianuju Ibifuro OJIKAH with Matric. No.: LCU/PG/000253 in the Department of Biological Science, Faculty of Basic Medical and Applied Sciences, Lead City University, is in full compliance with the approved university format and style.

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Signature Date