"EXPRESSION PROFILING OF RELN GENE IN SCHIZOPHRENIA"

By

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Dissertation Submitted To

B.L.D.E. (DEEMED TO BE UNIVERSITY), VIJAYAPURA, KARNATAKA

In partial fulfillment of the requirements for the degree of

Master of Science

In

Medical Biochemistry

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ACKNOWLEDGEMENTS

This piece of work has been accomplished with the grace of almighty God. It gives me great pleasure to express my heartfelt gratitude to everyone who has helped me, directly or indirectly, explore the horizons of knowledge.

I express my profound gratitude and sincere thanks to my guide, Dr. Nilima Dongre, Professor, Department of Biochemistry, BLDE (DU), Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura, for her constant encouragement and unfailing support, professional insight, valuable suggestions, motivation and exemplary guidance to carry out and complete this dissertation. I am deeply grateful to her for providing me necessary facilities and excellent supervision to complete this work.

I acknowledge my gratitude to Dr. Shivakumar P. Chaukimath, Professor; Dr. Santhosh Ramdurg, Associate Professor; and Dr. Sreerag Ashok and Dr. Varinder Bajaj, Postgraduates, Department of Psychiatry, BLDE (deemed to be university), Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura, for their support, advice, and help in data collection.

My Sincere thanks to Dr. B. B. Devaranavadagi , Professor and Head, Dr. B. S. Aski, Professor, Dr. Deepa Sajjanar, Dr. Indira Hundakeri, Associate Professor, Dr. S. S. Walvekar, Dr. Shivaleela Biradar, Dr. R Chandramouli Reddy, Assistant professor, Mr. Govindanagouda Naregal, and all non-teaching staff, Department of Biochemistry and Dr. G. S. Kadakol, Genetics Laboratory, Department of Anatomy, Dr. Aravindgouda Patil, Assistant Professor, Allied Health Science BLDE (DU), Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura, for their help, valuable suggestions and encouragement which has helped me to improve my research work.

I extend my thanks to PhD Scholars and postgraduate colleagues for their cooperation during the preparation of this dissertation

I sincerely thank Dr. Aravind Patil, Principal, BLDE (DU), Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura, for constant support and inspiration.

I thank Dr. Vijaya Soraganvi and Mr. Muragesh Math, Statisticians, for their masterly guidance and statistical analysis. I sincerely acknowledge the support and kindness shown

towards me by Dr. Prasanna Kumara B.M, Chief Librarian and Mr. Somashekar Assistant Librarian , also the staff of Central Library. and Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura, at all times.

My heart felt thanks to my beloved parents, Mr. Savalegeppa Shattar and Mrs. Jayshree Shattar, and my sisters, Miss. Soumya Shattar, Miss. Shweta Shattar and Miss. Payal T. R.

Last but not least, my sincere thanks to all the participants who volunteered for this study and rendered their cooperation, without which this study would not have been possible.

LIST OF ABBREVIATIONS

WHO	–	World Health Organisation
SCZ	–	Schizophrenia
MDD	–	Major depressive disorder
OCD	–	Obsessive compulsive disorder
D_2 receptor	–	Dopamine receptor
ICD 10	–	International classification of disease edition –
DSM – IV	–	Diagnostic and Stastiscal manual of mental disorder edition - IV
CIA	–	Clinical impairment assessment
PANSS	–	Positive and negative symptoms scale
BPRS	–	Brief psychiatric rating scale
APA	–	American psychiatric association
SGAs	–	Second generation antipsychotics
FGAs	–	First generation antipsychotics
M_3 Receptors	–	Muscarinic acetylcholine receptor
$5 – HT_{2A}$	–	5-Hydroxytryptamine 2A receptor
$5 – HT_{2C}$	–	5-Hydroxytryptamine 2C receptor
RELN	–	Reelin gene
SNPs	–	Single nucleotide polymorphism
APOE2	–	Apolipoprotein E receptor 2
VLDLR	–	Very low density lipoprotein receptor
DAB1	–	Disable1
GABA	–	Gama – aminobutyric acid
NMDA receptor	–	N-Methyl- D-Aspartate receptor
MHC	–	Major histocompatibility complex
Rl	–	Reeler
ORF	–	Open reading frame
YAC	–	Yeast artificial chromosome
FISH	–	Florescence in situ hybridization
ECM	–	Extracellular matrix
DLPEC	–	Dorsolateral prefrontal cortex

APOE	–	Apoprotein E
MD	–	Mood disorder
BMI	–	Body mass index
CTR	–	C- Terminal region
$(GAD)_{67}$	–	Glutamate decarboxylic acid
HDL	–	High density lipoprotein
LDL	–	Low density lipoprotein
VLDL	–	Very low density lipoprotein
CPZ	–	Chlorpromazine
CZP	–	Clozapine
Mets	–	Metabolic syndrome
IDF	–	International diabetic federation
WC	–	Waste circumference
NCEP - ATP - III	–	National cholesterol education programme - adult treatment panel – III
AHA	–	American Heart Association
RBS	–	Random blood sugar
RNA	–	Riboxynucleic acid
DNA	–	Deoxyribonucleic acid
TRI reagent	–	Trizol reagent
RT – PCR	–	Real time polymerase chain reaction

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CONTENTS

SL. No	Contents	Page No
1	Introduction	1-9
2	Review of literature	10-24
3	Aim and Objectives	25-26
4	Material And Methods	27-43
5	Results	44-62
6	Discussion	63-68
7	Conclusion Summary & Limitations	69-70
8	Bibliography	71-81
9	Annexure	81

LIST OF TABLES

Table No	Title	Page No
1	Classification of Schizophrenia	2
2	Neurodevelopmental Disorders and its findings	18
3	No. of individual in study group and control group	45
4	Clinical and Demographic profile of all subjects.	46
5	Distribution of study group on the basis of BMI	47
6	Mean±SD of biochemical parameters in the study group and control group	48
7	Mean±SD of SCZ group and control group	49
8	Bivariate correlation between BMI and Biochemical parameters in SCZ group	50
9	Bivariate correlation between WC and Biochemical parameters in SCZ group	52
10	RT-PCR analysis results value of study group and control group	57
11	Bivariate correlation between RELN gene expression level with BMI, WC and Biochemical parameters in SCZ group	59

LIST OF FIGURE

Figure No	Title	Page No
1	Cytogenetic Location of chromosome 7q22	9
2	Schematic Representation of primary sequence of RELN protein	17
3	Graph showing clinical and demographic profile of all subjects	46
4	Graph showing grading of patients on the basis of BPRS	47
5	Graph showing lipid ratios in study group and control group	48
6	Graph showing a various antipsychotics drugs	49
7	Graphs showing bivariate correlation between BMI and biochemical parameters SCZ group	50-52
8	Graphs showing bivariate correlation between WC and biochemical parameters SCZ group	53-55
9	Graph showing an expression of RELN gene	59
10	Graphs Showing Bivariate correlation between RELN gene expression level with BMI, WC, and Biochemical parameters in SCZ group	60-62

LIST OF ANNEXURE

Annexure No	Title	Page No
1	Plagiarism Verification Certificate	82
2	IEC- Approval Form	83
3	Proforma	84
4	Assessments of symptoms of psychotic disorder	85
5	Consent Form	86
6	MASTER CHART – a) Study Group b) Control group	87-88

ABSTRACT

Background:

Schizophrenia (SCZ) is a devastating neuropsychiatric condition of uncertain ethology with significant adverse effects on affected people, their families, and society. Heterogeneous population is seen in India with high degree of inbreeding. Hence it is necessary to screen the Indian psychotic patients in order to get a true picture of contribution of RELN mRNA expression level in SCZ. Mental illness is a leading cause for several metabolic changes and other related complications. It is not clear these metabolic changes may be due to alterations in the RELN gene expression or which may be because of the use of antipsychotic drugs. It is necessary to study the link between the RELN gene expression and metabolic syndrome.

Aim & Objective:

The present study aims to see the expression profiling of RELN gene in SCZ patients and to find the occurrence of metabolic syndrome in the them.

Methodology:

The clinically diagnosed patients with Schizophrenia and other mental disorders were studied for the RELN gene expression and quantification of the RELN protein using RT PCR and western Blotting. The Biochemical parameters like Serum RBS, Lipid profile were analysed by standard biochemical methods on the Semi autoanalyzer and lipid ratios were calculated in the study groups and compared with the age and sex matched controls. The statistical analyses were performed using a statistical package for the social sciences (SPSS) (Version 20). $p<0.05$ was considered statistically significant. All statistical tests performed were two-tailed.

Results :

The data shows 50% participants suffered from SCZ and remaining suffered with other psychotic disorders. The maximum no of participants showed moderate score based on BPRS Scale. The levels of expression of RELN gene were decreased in SCZ. The levels of RBS, TC, TG, & LDL-C were significantly increased in the SCZ patients as compared with the controls. The levels of HDL-C were significantly decreased in them. The BMI, WC and Lipid ratios also found to be significantly increased in the SCZ patients. About 54.5% females and 45.5% males were found be prone for developing the metabolic syndrome.

43.2% participants were on Clozapine, 111.7% on Olanzapine, 18.3% on Risperidone and 18.3% on Quetiapine.

Conclusion :

Genetic analysis of the candidate genes in the psychotic disorders will be helpful in designing the therapeutic drug target drugs for their treatments. Early and regular monitoring of the patients on the antipsychotic drug treatments must be done to find and prevent risk of the patients to develop the metabolic syndrome which is the major leading cause for atherosclerotic cardiovascular diseases in these patients. The life style modifications and early interventions will help in preventing the early deaths in the psychiatric patients.

Keywords: Schizophrenia, RELN Gene, RT-PCR, Expression Analysis**.** Glucose Level, Lipid Profile,

Introduction

Chapter - I

1. Introduction :

Mental illness can be defined as a change in mood, behavior, or thought patterns that impair daily functioning and cause suffering. Temporary mental health issues that are milder forms of mental disease might be brought on by everyday stress. The lack of awareness of these mental health issues might lead to serious mental diseases. The World Health Organization (WHO) estimates that more than 450 million individuals worldwide suffer from mental diseases. These are typical in nations with poor and medium incomes. Numerous epidemiological studies conducted in India revealed various prevalence's ranging from 9.54 to 370 per 1000 people. A person may have Major Depressive disorder (MDD), Anxiety, Obsessive-compulsive disorder (OCD), Phobia, or Other mental illnesses: Eating disorders, Manic-depressive disorder, Bipolar disorder (BPAD), Schizophrenia (SCZ), and Personality disorders. Genetic factors, trauma, child abuse, violence, social isolation, loneliness, prejudice, homelessness or substandard housing, and unemployment are a few of the causes of mental health issues. [1]

1.1 Schizophrenia :

Schizophrenia (SCZ) translates as **"divided mind".** It is found that mainly the young adults are affected by this neurodevelopmental disorder. The symptoms mainly include cognitive and emotional disturbances, including negative (such as aversion, alogia, apathy, poor or non-existent social functioning) and positive (such as delusions and hallucinations) symptoms. [2]

1.2 Classification of Schizophrenia :

Table No. 1 - Classification of Schizophrenia

Paranoid	Suspicious of others, Delusions of grandeur, Hallucinations
Disorganized	Speech is Disorganised and hard to follow, Inappropriate moods, No Hallucinations
Catatonic	Withdrawn, Isolated, with Very little physical movement, May assume unusual body positions.
Schizoaffective	A mixture of schizophrenia and affective disorders such as depression.
Residual	Low-level positive symptoms, Still psychotic symptoms.
Undifferentiated	Does not fit into any of the five categories.

1.3 Prevalence of Schizophrenia:

According to WHO estimates, schizophrenia (SCZ) affects approximately 24 million people worldwide. Schizophrenia is more common in some places, including in different countries and local communities. The median incidence of schizophrenia fluctuates between 0.15 and 0.20 per 1000 people per year, according to the most current publications, and it is greater (7 per 1000 people) in the 15 to 35-year-old age group. It results in about 1% of disability-adjusted life years globally. There are significant gender differences among those who have schizophrenia. Men typically exhibit more negative symptoms than women, who show more effective signs. [3,4]

1.4 Stages of SCZ :

Women have more extended social roles to play before the disease, which could be one cause for the illness's older age of onset. Another factor could be how estrogen affects the central nervous system's Dopamine (D2) receptors, which are less sensitive. Patients with schizophrenia frequently struggle to categorize sensory input. However, they may also perceive noises, colors, and other environmental elements more vividly than average. If left untreated, most SCZ gradually distance themselves from social contacts and lose the ability to care for their requirements and appearance. [5]

Three stages can describe the course of schizophrenia in adults –

1.4.1 Acute Stage :

- The patient's loss of reality is overt during the acute stage. (psychotic episode) that calls for assistance and care.

1.4.2 Stabilisation Stage :

- The early psychotic symptoms have subsided during the stabilization stage. The patient's condition has been managed, but a relapse is possible if there is a break in treatment.

1.4.3 Maintenance Stage:

- The patient is comparatively stable and can communicate throughout the maintenance stage. Be kept on antipsychotic drugs indefinitely. [6]

1.6 Diagnosis and Clinical Symptoms of Schizophrenia :

The two primary systems used to diagnose schizophrenia are the tenth edition of the International Classification of Diseases (ICD-10) and the fourth edition of the Diagnostic and Statistical Manual (DSM-IV). In contrast to ICD-10, the DSM-IV system needs social or occupational dysfunction and a six-month illness duration as opposed to one month under ICD-10.

Three main categories of symptoms are described in schizophrenia:

- Psychotic or positive symptoms
- Negative Symptoms
- Cognitive dysfunction [7]

1.6.1 Positive or Psychotic Symptoms –

Psychotic symptoms can be understood as defeat in contact with reality. People show unusual and weird behavior toward others. For three people, these symptoms come and go, and for others, these can be stable over time. It includes :

Hallucinations :

Hallucinations are sensory impressions that happen without any external cause. Any stimulation that triggers them occurs inside the sick person's brain, not in the outside environment. Any sense, including sight, sound, taste, smell, and touch, can experience genuine hallucinations.

a) Visual hallucinations.
b) Auditory hallucinations.
c) Olfactory hallucinations.
d) Somatic or tactile hallucinations. [8]

a) **Visual (sight) hallucinations:** These hallucinations involve perceiving unreal objects, figures, people, animals, or lighting.

b) **Auditory hallucinations :**

The most frequent kind of hallucinations is those involving sound or hearing. They comprise imagined sounds like music, footsteps, or slamming doors. Even in silent situations, some people may hear voices. Voices can be unbiased, antagonistic, or supportive. They might offer you orders that put you or others at risk.

c) **Olfactory (smell) hallucinations:** These hallucinations entail sensing smells that are either unreal or unattainable to anybody else.

d) **Hallucinations involving touch or touch sensations:** These hallucinations make you feel like someone is touching you or moving inside you when it isn't happening. They could make you feel like your internal organs are moving around or like bugs are crawling on your skin. [9]

Delusions :

a) **Persecution delusions:** The belief that someone is being pursued by anyone, occasionally an indeterminate "they." These psychopathological delusions usually involve unusual assumptions and plans, such as the idea that Martian aliens are attempting to poison me by injecting radioactive substances into my water from the tap.

b) **Confusion over the context:** The premise is that every individual and every event, regardless of context, has a unique and essential meaning. People with schizophrenia may think that a commercial or a TV character is speaking to them directly.

c) **Delusions of grandeur:** The idea that one is a famous or significant figure, such as Napoleon or Jesus Christ, is known as having delusions of grandeur. Another facet of delusions of grandeur is the belief that one has unique powers that no one else does, such as the ability to fly.

d) **Delusions of control** are the conviction that unnatural, external forces are in control of one's thoughts or behavior. There are other common delusions of control, such as thought broadcasting (the belief that others are receiving my private thoughts), thought insertion (the impression that someone is putting ideas into my head), and thought withdrawal (the assumption that the Clinical Impairment Assessment (CIA) is depriving me of my thoughts).

e) **Disorganized behavior:** Unusual behaviors such as repeated ineffective movements or excessive giggling (**keeps laughing in a childlike manner**)

f) **Disorganized speech:** Rambling, showing loose association, making illogical statements that reflect disorganized thoughts. [10]

1.6.2 Negative Symptoms :

One of the unfavorable signs of schizophrenia is a lack of or a reduction in several certain activities and functions. Chronic morbidity and poor functional outcomes of schizophrenia patients are primarily due to negative symptoms. A significant difficulty for businesses is treating negative symptoms. Negative symptoms, as defined by measures such as the Positive and Negative Symptom Scale (PANSS), the Rating of Negative Symptoms Scale (SANS), The Brief Psychiatric Rating Scale (BPRS), and the energy factor compare first- and second-generation antipsychotic medication therapies. [11]

1.6.3 Cognitive impairment :

Today, cognitive impairment is recognized as one of the fundamental characteristics of schizophrenia. All patients have been demonstrated to exhibit cognitive impairment in various areas, including verbal learning and memory, visual learning and memory, verbal comprehension, verbal processing speed, and working memory. In schizophrenia, the degree of positive and negative symptoms has consistently been found to be less important than the severity of cognitive impairment in predicting poor functional outcomes. [12]

1.7 Causes of Schizophrenia :

Inadequate knowledge of the origins of schizophrenic disorders is one of the factors contributing to the continuous difficulty in categorizing these disorders. These illnesses are believed to be the outcome of genetic, neurological, and environmental factors. The condition may be related to high amounts of dopamine, a brain chemical that carries messages, according to a leading neurobiological theory (neurotransmitter). [13]

Stress, either during pregnancy or at a later stage of development, is increasingly being identified as one of the environmental causes of schizophrenia. Stress is thought to cause schizophrenia by causing the body to produce more of the hormone cortisol. It is generally accepted that among those genetically predisposed to developing schizophrenia, those exposed to a very critical or stressful environment have a higher propensity to do so than those not. A stressful event in the sufferer's life frequently causes the condition to start. In a

susceptible person, the loss of a loved one, a painful failure, rejection, or disappointment can trigger schizophrenia. Therefore, it can be observed that schizophrenia and Intense external stress, family pressure, and social pressure can harm a vulnerable person and cause schizophrenia due to biochemical abnormalities in the brain. Families with a genetic history of sickness are more at risk. [14]

1.8 Treatment of Schizophrenia :

Treatment goals for schizophrenia include targeting symptoms, controlling degeneration, and improving adaptive functioning to facilitate the patient's reintegration into society. Patients have a slim chance of regaining their previous degree of adaptive function. Therefore, it is necessary to use both pharmaceutical and non-pharmacological therapies to maximize long-term results. Pharmacotherapy plays a significant role in managing schizophrenia, but residual symptoms may persist. For that reason, non-pharmacological treatments, similar to psychotherapy, are further necessary. The American Psychiatric Association (APA) suggests atypical antipsychotics or Second Generation antipsychotics (SGAs) as the first-line therapy for schizophrenia, except clozapine. Since SGAs are less frequently linked with side effects, they are typically preferable over traditional antipsychotics First Generation antipsychotics (FGAs). Other symptoms. However, the adverse metabolic effects of SGAs include weight gain, hyperlipidemia, and diabetes mellitus. These negative consequences might raise schizophrenic patients' risk of cardiovascular mortality. [15]

Antipsychotic Drugs and Metabolic Syndrome :

Antipsychotic medications are primarily and widely prescribed to treat schizophrenia and other psychiatric conditions such as bipolar disorder, depression, dementia, and drug addiction. Traditional or first-generation antipsychotic medications like haloperidol, perphenazine, and chlorpromazine work by blocking the dopamine (D2) neuroreceptor. Their ability to bind with dopamine (D2) neuroreceptors determines the profile of their adverse effects. Sedation, anticholinergic effects, extrapyramidal symptoms, hypercholesterolemia, agranulocytosis, metabolic syndrome-related problems, etc., are a few of the negative effects of antipsychotic medications. [16] Second-generation antipsychotics typically referred to as atypical antipsychotics, possess clozapine, olanzapine, and

risperidone. They block some neuroreceptors, including the dopamine-independent 5-HT2A/5-HT2C, H1, and muscarinic acetylcholine receptor (M3) receptors. [17]

Atypical antipsychotics are frequently recommended due to their higher tolerability and decreased risk for additional adverse effects compared to first-generation antipsychotics, according to previous studies, and they are especially effective in treating schizophrenia. But compared to typical antipsychotics, atypical antipsychotics are recognized to carry a higher risk for harmful metabolic effects. These medications raise the possibility of metabolic disorders, including weight gain, obesity, hyperglycemia, dyslipidaemia, and diabetes. Diabetes is more prevalent and 2 to 3 times more prevalent in schizophrenia patients, and it is mediated by insulin resistance and co-administered antipsychotics. Additionally, even when used for a brief time, both standard and atypical antipsychotics were linked to weight increase. The primary outcome of weight growth was connected to conventional and atypical antipsychotics, even when administered briefly. Atypical antipsychotics' main weight-related side effect is on adipose tissues, which increases insulin resistance, glucose intolerance, and diabetes mellitus. Without causing more weight gain, SGAs are also connected to hyperglycemia and diabetic ketoacidosis. The type of atypical antipsychotic used determines the incidence of metabolic problems.. [18]

1.9 Reelin (RELN) Gene and Schizophrenia :

Schizophrenia has a significant genetic component, with a heritability of approximately 80%. The Schizophrenia Working Group of Psychiatric Genomics examined single nucleotide polymorphisms (SNPs) and copy number variations as a complement to schizophrenia genetics in genome-wide association studies. According to some findings, schizophrenia may be caused by multiple functional differences in genes in neurodevelopmental pathways. [19]

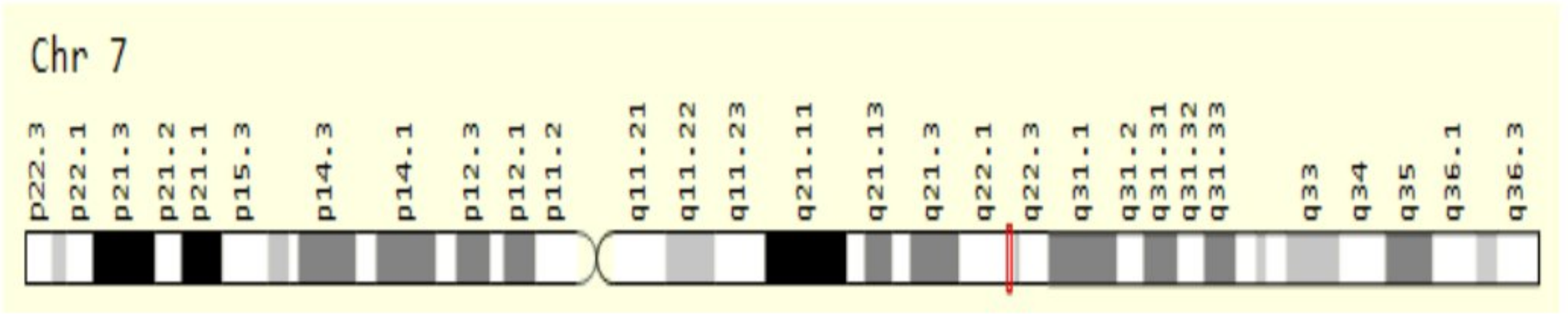
. During earlier products, *RELN*, an extracellular matrix glycoprotein, plays a role in migrating neuronal cells and the lamination of corticolimbic structures. Additionally, it encourages dendritic formation, protein translation, and spinal development in postnatal life. *RELN* regulates neuroplasticity and functioning in adults and contributes to the development of the hippocampal neurons. Apolipoprotein E receptor 2 (ApoE R2) and the very low-density lipoprotein receptor are activated by *RELN*, which has biological consequences

(VLDLR). The adaptor protein Disable-1 is phosphorylated by the *RELN* signal, which also activates Src/Fyn kinases (Dab1). [20,21]

The possible part *RELN* plays in developing neurodevelopmental disorders, including schizophrenia. Thus, potential genes for schizophrenia include genes encoding *RELN* and proteins implicated in the signaling pathways of *RELN*.

The Reelin *(RELN)* gene cytogenetic position on chromosome 7q22. [22]

Fig. No. 1 – cytogenetic Location of chromosome 7q22



Review of Literature

Chapter – II

2. Review of Literature :

In this literature review, the meaning of the term "schizophrenia" and a brief history of how the term "schizophrenia" came to be will be covered. The literature's primary focus will be on psychiatric care, specifically mental institutions and their effects on the well-being of people admitted for the first time for psychotic symptoms. The expression of the Reelin (RELN) gene in schizophrenic patients is another important literary topic.

2.1 Schizophrenia (SCZ) :

Schizophrenia (SCZ) is a devastating neuropsychiatric condition with significant adverse effects on affected people, their families, and society. Onset is very rare in early childhood. Usually, it is a middle age onset disease; for males, the peak age of onset is 20-28 years, and for females, it is 26-32 years. [23]

2.1.1 Prevalence / Incidence of schizophrenia :

Systematic evaluations show that SSCZ is more prevalent though incidence is less may be because it frequently manifests in early adulthood and progresses to chronic disease. The incidence of schizophrenia varies demographically According to a study, it accounts for 2.8% of years lived with disability and 1% of total disability-adjusted life years globally. [24]

2.1.2 Etiology of schizophrenia :

Here, the function of the family in the etiology of schizophrenia is studied, along with the sense of sadness brought on by a diagnosis of schizophrenia and the debates surrounding expressed emotion. The family is a source of genetic material and an environmental supplier. Additionally, research is done on the function of the family in the treatment of schizophrenia as well as its involvement in advocacy and service promotion. Our observation is still accurate by considering the many roles families play in schizophrenia. [25]

2.1.3 Pathophysiology of Schizophrenia :

Three main ideas exist about the progression of schizophrenia. Dopamine, serotonin, glutamate, and gamma-aminobutyric acid (GABA) imbalance is thought to be the primary contributor to the disease's mental symptoms, according to the neurochemical abnormality theory. It implies that the onset of schizophrenia may be influenced by the four main dopaminergic pathways. This dopamine hypothesis proposes that the positive symptoms of

the condition are due to increased D2 receptor activation in the mesolimbic pathway. Low dopamine levels in the mesocortical region, which in turn causes the unpleasant symptoms of the condition, are thought to be caused by the mesocortical pathway. Higher prolactin levels brought on by decreased tuberoinfundibular dopamine availability due to obstruction of the tuberoinfundibular route may cause other symptoms including amenorrhea and a diminished libido. Despite the fact that serotonergic hyperactivity has also been linked to schizophrenia [26]

2.1.4 Diagnosis of Schizophrenia:

According to the Diagnostic & Statistical Manual of Mental Disorders, 5th Edition (DSM-V), The first episode of psychosis often happens in the early years of adulthood. Positive, negative, and cognitive symptoms are all experienced by patients with schizophrenia. Delusions, hallucinations, and disorganized speech are examples of positive signs. Poor speaking and a flat mood are examples of negative symptoms. Some cognitive symptoms are attention, working memory, and executive functioning (DSM-V). Many of these symptoms impact a patient's functional independence and frequently cause social and/or vocational problems.[27, 28,29),

In addition, several studies have demonstrated that of SCZ patients are altered due to changes in gene expression. Potential therapeutic targets for SCZ include the cys/glu antiporter system xc, which promotes glutaminergic neurotransmission by releasing glutamate into synapses. (27,28).

Despite the fact that dopaminergic hypofunction in the frontal brain and dopaminergic hyperfunction in the limbic system are both implicated in the pathophysiology of schizophrenia, many aspects of the aetiology of SCZ remain unknown. [30,31,32] There is emerging understanding that immunological dysregulation may contribute to neurodevelopmental disorders with hereditary components like schizophrenia. [33,34] In the major histocompatibility complex (MHC) region on chromosome 7, there is growing evidence for a strong association between schizophrenia and genes that control brain development. This association may explain the abnormalities in cognition, behaviour, and brain structure seen in psychotic disorders..[35,36]

2.2 Reelin *(RELN)* Gene :

Reelin, a glycoprotein encoded by the RELN gene, is generated by particular kinds of developing brain cells and triggers a signaling cascade in postmitotic migratory neurons that is necessary for the appropriate placement of neurons within layered nervous system parenchyma.[37]

2.2.1 Cloning and Expression :

Reeler (rl), an autosomal recessive mouse mutation, causes ataxia, tremors, and poor motor coordination. The structure of the cerebellum and cerebral cortices as well as other membrane areas is disrupted in afflicted mice because neurons are unable to go to their intended sites in the developing brain.

In 1995, D'Arcangelo et al. identified the reelin *(RELN)* gene, which was deleting in two-reeler alleles. Transgene insertion was employed to create the allele that was used to clone the gene. Reelin was expressed in embryonic and postnatal neurons during times of neuronal migration in normal mice but not in mutant ones.. A putative polyadenylation signal and around 1 kb of 3-prime translated sequence after the stop codon. A protein with 3,461 amino acids and a relative molecular mass of 388 kD is encoded by the ORF. The RNA from the brains of unaffected mice did not contain any reelin transcripts, whereas the brains of normal mice had one 12 kb reelin transcript. [38,39,40]

Human reelin (RELN), like its murine cousin, is important and encodes an mRNA that is about 12 kb in size, according to DeSilva et al. (1997). The ORF of the overlapping cDNA clones predicted human and mouse proteins with 94.2% and 87.2% identity in terms of amino acid and nucleotide sequences, respectively, and similar sizes (388kD). A northern hybridization study revealed that RELN is expressed in the liver, foetal, and postnatal brain in addition to other organs. RELN expression was high in the cerebellum of the postnatal human brain..[41]

2.2.2 Gene Structure:

The genomic organization of the mouse *RELN* gene and the 5-prime-flanking genomic DNA sequences were characterized by Royaux et al. in 1997. The gene's 450 kb of genomic DNA is divided into 65 exons. By using two separate polyadenylation sites and alternate splicing of a micro exon, they were able to identify several reelin transcripts. Except for the splice

donor site of intron 30, which is GC instead of GT, all splice sites follow the GT-AG norm. Intron 42 included a pseudogene that had been processed. Its nucleotide sequence shared 86% of its base pairs with the rat RDJ1 cDNA, which codes for a protein that is similar to DnaJ and belongs to the Hsp40 family. The genomic organization of the RELN genes in both mice and humans appears to be very similar.

Due to tandemly repeated areas in the reelin protein, it was hypothesized that gene duplication events occurred throughout evolution. Royaux et al. (1997) proposed a scenario for developing the reelin gene's repeat coding section from a possible ancestral minigene based on a comparing the amino acid sequences of the 8 repeats and the placements of introns.[42]

2.2.3 Mapping :

The gene mapping used a mouse reelin probe to separate human cDNA from a cerebellar phage library to locate the RELN gene. After that, fluorescence in situ hybridization (FISH) was performed using a P1 clone. The human RELN gene is located on 7q22, a part of the chromosome that has not yet been connected to any hereditary disorders in people. The 7q22 region of Yeast Artificial Chromosome (YAC) contigs was also used to map RELN. The mouse RELN gene is located on chromosome 5, which is known to share a significant area with human chromosome 7. The RELN gene was assigned to chromosome 7q22 based on localization within a suitable YAC contig and both Fluorescence In Situ Hybridization (FISH) and localization.[43,44,45,46]

2.2.4 Functions of RELN gene :

Throughout the course of brain development, reelin regulates the positioning and/or tropism of Purkinje cells, interneurons, and cortical pyramidal neurons. Impagnatiello et al. (1998) assert that this implies that RELN may contribute to schizophrenia. (47) Another element that is essential in guiding the migration of embryonic cortical neurons to their final resting locations in the subcortical plate is the mouse gene mutation (Dab1). Another element that is essential in guiding the migration of embryonic cortical neurons to their final resting locations in the subcortical plate is the mouse gene mutation (Dab1). The extracellular matrix (ECM) proteins that the RELN protein interacts with are hypothesised to be the source of a signalling cascade that phosphorylates the adapter protein (Dab1) produced by this gene [50] .

Impagnatiello et al. (1998) examined the post-mortem prefrontal, temporal, hippocampal, caudate, and cerebellar cortices of schizophrenia patients and their matched nonpsychiatric controls. RELN and its mRNA were considerably downregulated (by around 50%) in all brain regions examined in individuals with schizophrenia; this reduction was also present in patients with undifferentiated or paranoid schizophrenia. However, in all these regions where RELN was decreased, DAB1 expression was normal. The prevalence of RELN DNA polymorphism in schizophrenia patients and the location of this variation in a section of genomic DNA critical for the control of RELN protein production sparked clinical interest in RELN gene abnormalities as potential schizophrenia risk factors.[51]

According to **Grayson et al. (2005),** postmortem brains from schizophrenia patients exhibited more methylation of the RELN gene in the promoter region than controls, notably at locations -134 and -139. The authors proposed that lower expression of RELN in schizophrenia is caused by hypermethylation of this promoter region.[52]

In **April 2018, Elisa Brietzke et al.** conducted a postmortem study. This study compares the expression of genes connected to the RELN pathway in the postmortem brains of people with schizophrenia (SCZ) and mood disorders (MD) with that of a healthy control group (HC) to determine whether there is a possible mediating effect from body mass index (BMI). 849 samples from the Dorsolateral prefrontal cortex (DLPEC) and 579 samples from the hippocampus are used in this investigation. This research found that group and BMI significantly influenced the expression of RELN, CAMK2A, CAMK2N2, and GRIN2A. Compared to HCs, the hippocampus of the person with MD had a decreased expression of Apolipoprotein (APOE). The findings of this study suggest that there may be differences in the expression of genes involved in the insulin pathway between a person with SCZ or MD and healthy control, with a higher BMI being associated with greater vulnerability.[53,54] .

2.2.5 Reelin *(RELN)* protein and gene architecture :

RELN is a large extracellular glycoprotein of approximate molecular mass of 388 kDa with serine protease activity. The primary sequence of *RELN* protein begins with a cleavable signal peptide of 25-27 residues at its N-terminus followed by an F-spondin like domain (spanning amino acids 28-190). [55]

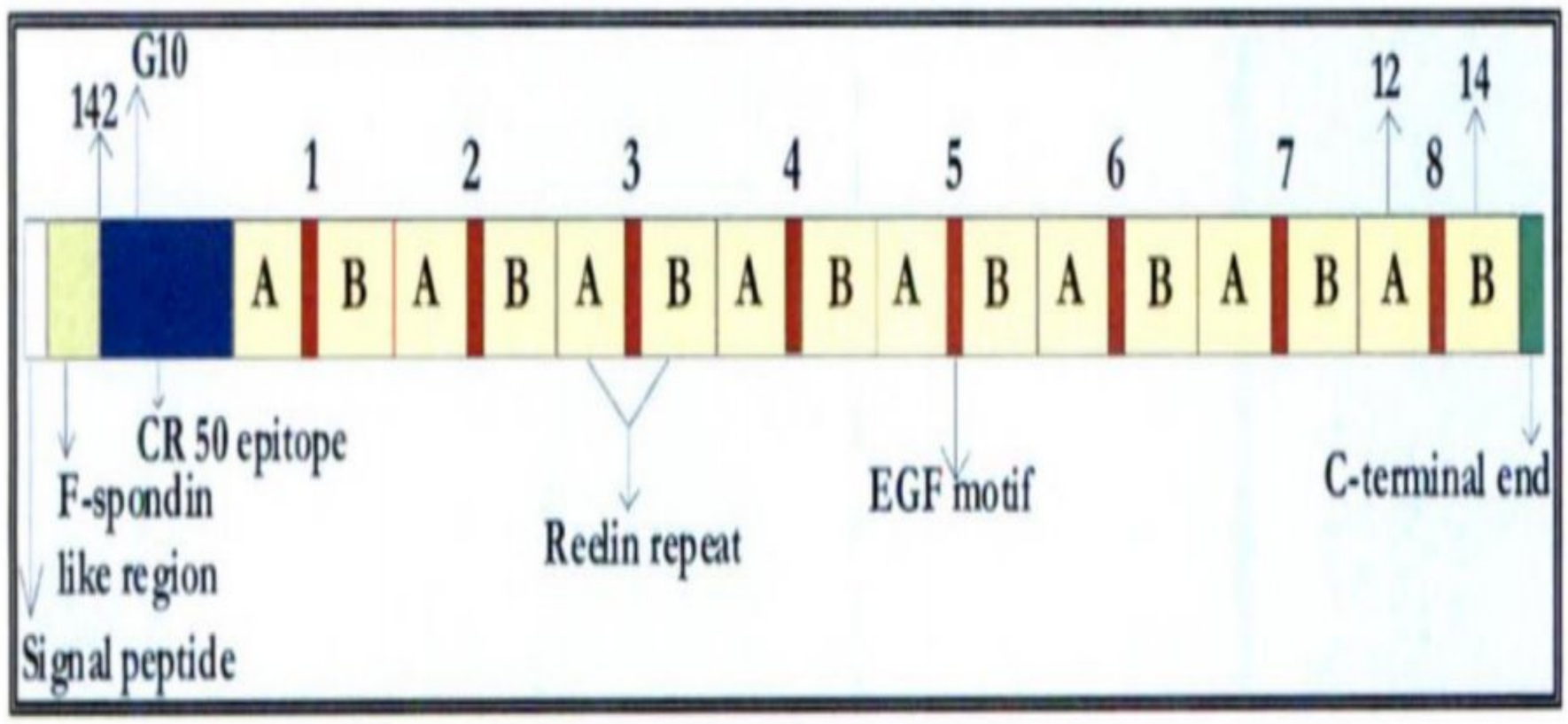


Fig. No. 2 Schematic Representation of primary sequence of Reelin protein

According to **Nakano et al., 2007**, the study has shown that the *RELN* C-terminal region's (CTR) exceptional conservation is required for *RELN* secretion and the activation of subsequent signalling processes. They demonstrated the *RELN* secretion in its wild-type (with CTR) and mutant (without CTR) forms, but only the mutant type displayed weak downstream signalling. Stabilizing the reelin-receptor connection caused by the interaction between positively charged CTR and negatively charged molecules on the neuronal membrane causes potent signal transduction.[56]

2.3 Reelin *(RELN)* and Neurodevelopmental Disorders :

A severe alteration in the cytoarchitectonics pattern formation of the brain caused by abnormal *RELN* expression and function in its signal transduction pathway may result in several neurodevelopmental disorders, including Schizophrenia, Bipolar disorder, Lissencephaly syndrome, Epilepsy, Autism, etc.[57,58,59,60,61,62]

Table No. 2 Neurodevelopmental Disorders and its findings

Neurodevelopmental Disorders	Findings
1. Schizophrenia	50% reduction of reelin mRNA and protein
2. Bipolar disorder	Reduction of reelin mRNA and protein
3. Lissenciphaly syndrome	Blood reelin is decreased
4. Epilepsy	Inverse correlation of reelin expression and granular cell dispersion (GCD) in human epileptic hippocampal tissues
5. Autism	Expression of reelin protein is reduced in brain and blood

One of the most researched aspects of schizophrenia is its genetics. However, the findings of several linkage analyses have not shed any light on the underlying etiological elements that determine the disease's symptomatology. But recent studies that looked at molecular markers that seemed to be abnormally regulated in postmortem brains of schizophrenia patients have provided some understanding. *RELN* and Glutamic acid decarboxylase $(GAD)_{67}$ have been shown to be the most aberrant among the over 100 distinct markers investigated in the setting of schizophrenia and bipolar disorder.[63]

The GABAergic neurons of the mammalian cortex specifically coexpress these two mRNAs and their related proteins. One of the more often verified results in the postmortem cortex of schizophrenia patients is the down-regulation of *RELN* and GAD67. We have previously noted that the postmortem brains of schizophrenia and bipolar patients have *RELN* mRNA and protein levels that are quantitatively decreased by 50% in various cortical areas.[64,65]

In the brains of schizophrenia patients, **Tochigi et al.(2008),** could not confirm their prior results that *RELN* expression is controlled by hypermethylation on the *RELN* promoter region. [66]

Several association studies have also been conducted to link the *RELN* gene polymorphism or expression to schizophrenia.

Akahane et al. 2002, investigated cytosine guanine guanine CGG repeats in the 5TJTR region of the *RELN* gene in schizophrenic patients and ethnically matched control samples but found no significant association.[67]

In a study conducted, **Chen et al. 2002,** discovered a single nucleotide polymorphism in the 5TJTR region of the *RELN* gene, but they were unable to find any positive association between this SNP and schizophrenia in a study of Chinese schizophrenic patients and control subjects. [68]

Although **Goldberger et al. 2005,** reported an association of CGG repeats in the 5'UTR region of the *RELN* gene in French Caucasian schizophrenic patients, Huang and Chen failed to replicate the same in a Chinese Han population from Taiwan. [69]

Shifman et al. 2008, demonstrate that a genome-wide correlation research found a common mutation, rs7341475 intron 4 of *RELN*, to be correlated with female schizophrenia patients and effectively reproduced the same finding in four other groups. (70)

In their latest study, **Gregorio et al. 2009,** found that schizophrenia patients with a non-synonymous Val997 Leu polymorphism (rs362691) of RELN had larger left and right ventricles.(71)

2.4 Treatment of Schizophrenia :

According to the literature, there are two primary categories of antipsychotic medications that can be used to treat schizophrenia. Typical or first-generation antipsychotics (FGAs), such as dopamine receptor antagonists (DRAs), and atypical or second-generation antipsychotics (SGAs), such as serotonin dopamine receptor antagonists (SDAs). (72)

2.4.1 Dopamine Receptor Antagonists (DRAs) :

The dopamine receptor antagonist (DRA) was the first effective class of drugs, developed in the early 1950s. Chlorpromazine (CPZ), which Delay and Deniker discovered, has shown promising effects in reducing schizophrenia symptoms. The discovery of the additional FGAs changed the way schizophrenia was treated. The first Serotonin Dopamine Receptor Antagonist (SDA), clozapine (CZP), was introduced in 1970. Later, more SDAs were created, which are now thought to be the primary medications for treating schizophrenia. For treating refractory schizophrenia, in particular, it was shown to be more effective than FGAs.(73)

2.4.2 Serotonin Dopamine Receptor Antagonists (SDAs) :

SDAs were classified as atypical antipsychotics due to their more expansive range of activities and lower propensity to produce extrapyramidal adverse effects compared to DRAs. All SDAs have been proven to have numerous neurotransmitter effects and complicated pharmacological actions. (74)

Leucht et al. 1999, observed that SDAs generally had greater tolerance when compared to DRAs. Even though its favourable benefits in many areas are highly recommended compared to DRAs, the most prominent adverse effects on metabolic parameters were increased weight gain, elevated blood pressure, diabetic Mellitus, and dyslipidemia. Before

considering atypical antipsychotics as a therapy, it should assess metabolic syndrome. SDAs should be used with extreme caution following a risk factor assessment and a baseline assessment for metabolic side effects.[75]

Despite the possibility that it might result in significant medical issues that increase morbidity and death, psychiatrists must treat schizophrenia. The use of antipsychotics to treat schizophrenia was based on a risk-benefit analysis of their potential side effects and therapeutic benefits. The symptoms of schizophrenia would get worse if a doctor didn't treat the patient due to metabolic problems and adverse effects from antipsychotics. A few studies also discovered that schizophrenia patients were more likely to develop metabolic syndrome than individuals who had never used drugs. [76]

2.5 Metabolic Syndrome (Mets) :

The term "metabolic syndrome" refers to a cluster of illnesses that increase the risk of atherosclerotic cardiovascular disease, insulin resistance, diabetes mellitus, and vascular and neurological consequences such as a cerebrovascular accident.

If a patient exhibits any three of the following, metabolic disarray is classified as a syndrome.

- Males with waist circumferences greater than 40 inches and females with waist circumferences greater than 35 inches
- More than or equal to 150 mg/dL or higher blood triglycerides
- Reduced high-density lipoprotein cholesterol (HDL) by less than 40 mg/dL for males or less than 50 mg/dL for women
- The elevated fasting blood sugar of at least 100 mg/dL
- Blood pressure levels of 130 mmHg or more in the systolic and/or 85 mmHg or more in the diastolic.

The effects of metabolic syndrome on a person's health and medical expenses are significant. It is important to acknowledge the increased incidence of metabolic syndrome in the world since treatment can potentially reverse or stop the illness's course.[77,79,80]

2.5.1 Etiology :

Metabolic syndrome is mostly caused by excess weight, obesity, inactivity, and hereditary risk. Adipose tissue accumulation and tissue malfunction, which result in insulin resistance,

are the main features of the condition. The increased adipose tissue releases proinflammatory cytokines that affect insulin processing negatively, including tumour necrosis factor, leptin, adiponectin, plasminogen activator inhibitor, and resistin. [81,82,83]

2.5.2 Epidemiology :

Adults in the United States over the age of 18 continue to have a considerable prevalence of metabolic syndrome. The incidence of this disease process grew by 35% between the 1980s and 2012, according to data. The incidence was reported to be 25.3% in the 1980s and rose to 34.2% in 2012. The National Health and Nutrition Examination Survey's (NHANES) most current data, however, indicates that the prevalence is declining, with 24% of men and 22% of women reporting it. [84]

2.5.3 Pathophysiology of Metabolic Syndrome :

Metabolic syndrome has an adverse effect on a number of body systems. Insulin resistance causes microvascular damage that increases a patient's risk for endothelial dysfunction, vascular resistance, hypertension, and vessel wall inflammation. Endothelial damage has the potential to disturb the body's equilibrium, resulting in atherosclerosis and the emergence of hypertension. Furthermore, hypertension affects several physiological functions by causing arterial stiffness and resistance to rise, which leads to peripheral vascular disease, structural heart disease, including left ventricular hypertrophy and cardiomyopathy, and renal impairment. Ischemic heart disease may also develop as a result of the metabolic syndrome's cumulative impact on endothelial dysfunction and hypertension. Blood can become thrombogenic due to endothelial dysfunction brought on by elevated levels of plasminogen activator type 1 and adipokines, and coronary artery disease might occur as a result of hypertension-induced vascular resistance. Additionally, metabolic syndrome-related dyslipidemia might accelerate the atherosclerotic process that results in symptomatic ischemic heart disease.[85]

2.5.4 Prevalence of metabolic syndrome in schizophrenia :

The high incidence of cardiovascular disease and diabetes mellitus, which increases the mortality rate, has recently focused attention on the prevalence of metabolic syndrome in schizophrenia.

An analysis of the literature reveals that **Almeras et al.** conducted the first prevalence research in Italy, where they calculated the prevalence of metabolic syndrome in

schizophrenia, which ranged from 11% to 33% depending on the antipsychotic medications used for schizophrenia.[86]

According to several cross-sectional studies, the incidence of metabolic syndrome among schizophrenia patients using antipsychotics ranged from 15% to 69%.[87]

According to a number of longitudinal investigations, the frequency of metabolic syndrome in drug-naive schizophrenia patients ranged from 0% to 14% at baseline, rising to 52.4% after three months of antipsychotic medication therapy. [88]

The prevalence of metabolic syndrome using IDF criteria was lower, as shown in two investigations conducted in general populations in south India using both the ATP III and IDF definitions of the condition. According to their reports, the prevalence was 41 and 25.8%, respectively. In contrast to this study, another Indian study of 227 schizophrenia patients found that the prevalence of metabolic syndrome was 44.5% when using modified National Cholesterol Education Program Adult Treatment Panel-III (NCEP ATP-III) criteria and 43.6% when using International Diabetes Federation (IDF) criteria. In a randomized, double-blind controlled, short-term prospective study conducted in India, schizophrenia patients had a five-fold greater prevalence of metabolic syndrome than the matching healthy control group. According to ATP IIIA and IDF criteria, the prevalence rates were calculated to be 10.1% and 18.2%, respectively. [89,90,-96.).

2.5.5 Predictors of Metabolic Syndrome :

According to a review of the literature, lifestyle variables including inactivity, a poor diet, and a high prevalence of smoking are among the risk factors for the development of metabolic syndrome in schizophrenia, along with hereditary factors and the inability to obtain general healthcare. [97] Antipsychotic medications have had a deleterious influence on the above modifiable risk factors for metabolic syndrome since the development of atypical antipsychotics. A substantial risk of developing metabolic syndrome and cardiovascular diseases are created by the cumulative long-term effects of poor general health, long-term antipsychotic medication exposure, and the prolonged nature of the illness. In those with first-episode schizophrenia, lower incidences of metabolic syndrome were seen. [98]

Pakkiyalakshmi N et al., (2018), Evaluated the growth of metabolic syndrome with the use of haloperidol and risperidone, as well as first episode schizophrenia patients who were followed up for 6 months, and discovered an 18.8% incidence of metabolic syndrome with

AHA (American Heart Association) criteria at the end of the 6 months. Haloperidol and risperidone groups did not differ in the rate at which the metabolic syndrome developed. [35]

According to NCEP ATP III criteria, [99]

Owusu-Ansah A et al. (2018), found that the total prevalence of schizophrenia patients in Ghanaians was 14.1%. This study also compared the prevalence between treatment-experienced and treated psychiatric patients and found that the majority of metabolic syndrome was higher in a group using atypical antipsychotics compared to typical antipsychotics (17.8% vs. 6.2% for schizophrenia patients receiving antipsychotic treatment). This study found that managing these patients requires ongoing monitoring of cardiovascular risk factors. [100]

Elena G. Kornetova et al. conducted a meta-analysis study in August 2019 to investigate weight changes and of body fat composition with glucose metabolism caused by restarting 2nd generation antipsychotics in patients with SCZ, whether they have MetS or not. The 114 (59M/55F) schizophrenia participants in this study range in age from 18 to 55. After six weeks of restarting medication, this study found no discernible alterations in the indices of glucose metabolism in patients with SCZ and MetS. In this patient's group, both the TC level and the atherogenic index had dramatically risen. Furthermore, changes in body fat composition and biochemical markers were examined depending on the medicine used, with the exception of seven individuals using clozapine due to limited sample size. After a 6-week course of treatment, there were no statistically significant changes in the markers of fat composition in the MetS patients on olanzapine, quetiapine, and risperidone. According to the study's findings, they showed for the first time through the monitoring of particular indicators that a number of body fat composition indices may rise even after only 6 weeks of SGA therapy. This can be a sign that individuals receiving SGA treatment need to be closely watched when they first start using it. Several MetS signs can also be reversed with good case management. [101]

Dahake HS et al. (2016), studied the uric acid level in patients with first-episode drug-nave schizophrenia, and chronic schizophrenia patients on atypical antipsychotic medication were examined. The results were determined with those of a healthy, age and sex-matched control group. This study showed a considerable reduction in uric acid levels in both first-episode and chronic schizophrenia patients compared to healthy controls, but no discernible

difference between first-episode and chronic schizophrenia patients. This study recommends lowering uric acid levels since it helps to combat reactive oxygen species in schizophrenia. [102]According to **Sankaranarayanan et al. (2013),** a cohort of patients attending a Clozapine clinic with a mean treatment duration of 79.5 months had a high prevalence of obesity and metabolic abnormalities. According to IDF criteria, 51% of patients had metabolic syndrome, indicating that SGA users had a higher chance of developing the condition and cardiovascular morbidity. The authors concluded that it is necessary to understand and monitor the use of atypical antipsychotics and life style factors underlying the risk of metabolic syndrome. . [104]

Specific Aims and Scope of the present study Research:

Reelin, a glycoprotein released by the first Cajal-Retzius neurons, plays a role in the migration and placement of newly formed neurons throughout brain development. Large deletions in the reelin gene cause a natural mutant mouse called Reeler, which exhibits severe neuroanatomical abnormalities and is used as a wonderful model for research on a variety of migration defect disorders and neurodevelopmental disorders, including schizophrenia, bipolar disorder, depressive disorder, and others.We choose schizophrenia and other psychotic disorders for genetic association analysis in the current study because RELN is a potential candidate gene for the illnesses.

So far, no genetic association studies on the reelin gene with schizophrenia and other psychotic disorders are known in the Indian population, despite a few recent papers from our research linking RELN with psychotic illnesses. This research study is covered in a few of these reports. To comprehend the risk posed by these markers, the primary goal of the current study is to investigate any potential genetic associations between RELN and the Indian population, as well as biochemical parameters like lipid profile and random blood glucose, using population- and family-based approaches.

The present study thus includes the following :

i. Analysis of the Reelin gene's (RELN) expression in schizophrenia and other psychotic diseases in the Indian population
ii. Case-control study to examine a potential link between metabolic syndrome in schizophrenia and other psychotic dise

Aim and Objectives

Chapter - III

Aims And Objectives :

Aims :

To determine the expression profiling of RELN gene in schizophrenia patients.

Objectives :

- To study the association of RELN gene in schizophrenic patients.
- To observe the relation between biochemical parameters like Lipid profile and Random Blood Sugar (RBS) with schizophrenia patients.
- To explore the possible role of RELN link for developing metabolic syndrome in patients.

Hypothesis :

Null Hypothesis :

There may not be any correlation of *RELN* gene expression in schizophrenia with metabolic syndrome.

Alternate Hypothesis :

There may be correlation of *RELN* gene expression in schizophrenia with metabolic syndrome.

Material and Methods

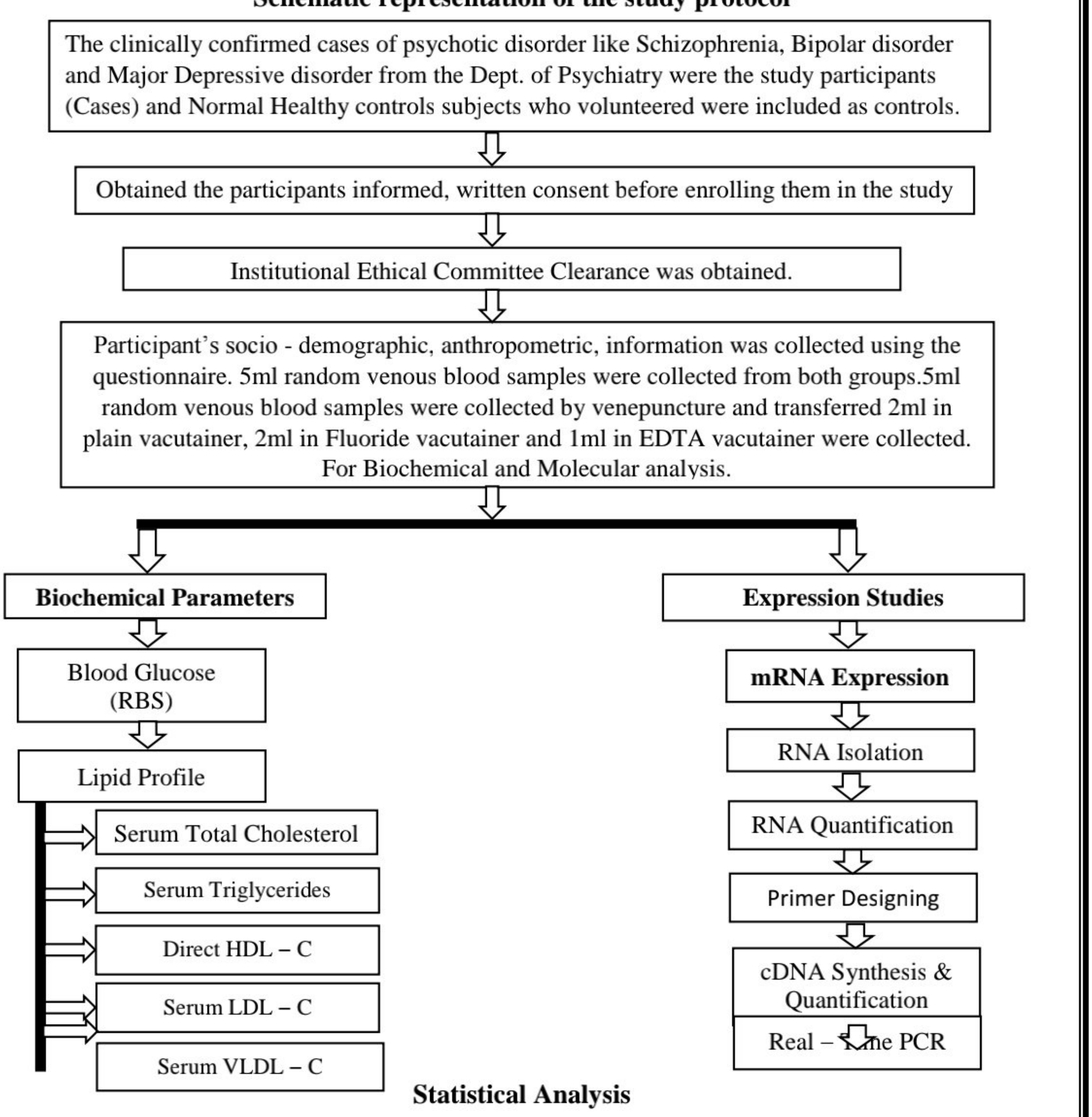
Chapter - IV

3. Materials and Methods :

The present study was conducted in Dept. of Biochemistry in Collaboration with Dept. of Psychiatry and Genetics Laboratory at BLDE (DU), Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura.

Biochemical and genetic association analysis of Reelin (*RELN*) gene markers with neurodevelopmental disorder such as Schizophrenia, Bipolar disorder, and Major depressive disorder is part of the current work. The following is a flowchart of the entire study process.

Schematic representation of the study protocol



Statistical Analysis

3.1 Ethical Clearance :

The study was ethically approved by Institutional Ethical Committee of BLDE (DU), **BLDE(DU)/IEC/718/2022-23, 30/08/2022** Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura.

3.2 Study Design : The present study is an analytical cross-sectional study in which a total of 108 samples were analysed over the course of six months. All subjects were in the age range of 20–65 years. After explaining the study to the patients, their voluntary consent was taken in writing.

3.2.1 Study Site : This study will be conducted in Dept. of Biochemistry in Collaboration of Dept. of Psychiatry and Genetics Laboratory, Centre for Advanced Medical Research (**CAMR**) at BLDE (DU), Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura.

3.2.2 Source of Data : This study will be conducted in our hospital ; outpatients who are coming for Psychotic symptoms in the department of Psychiatry.

Sample Size – Total Sample Size : 108

t tests – Means : Difference between two independent means (two groups)

Analysis : A priori: Compute the required sample size

Input :	Tail(s)	=	Two
	Effect size d	=	0.5450099
	α error prob	=	0.05
	Power (1-β err prob)	=	0.80
	Allocation ratio N2/N1	=	1
Output :	Noncentrality parameter δ	=	2.8319545
	Critical t	=	1.9825973
	Df	=	106
	Actual power	=	0.8012929
	Sample size group 1	=	54
	Sample size group 2	=	54
	Total sample size	=	**108**

Using G*Power ver. 3.1.9.4 software for sample size calculation, [Ref] Joshi kb, et al. cardiovascular disease risk in schizophrenia patients. A Case-Control Study. J Clin of Diagn. Res. 2013 ; 7(12) : 2694-2696. HDL Cases (Mean=36.8, SD=9.5) and HDL Control (Mean=42.2, SD=10.3), this study requires a sample size of 108. So to achieve a power of 80% for detecting a difference means t-tests - Means: Difference between two independent means (two groups).

3.3.3 Inclusion Criteria :

- Patients Diagnosed as having schizophrenia based on International Classification of Disease 10 (ICD -10) & DSM V Criteria.
- Patients with other than psychotic symptoms such as Bipolar disorder, Major Depressive disorder were included in the study.

3.3.4 Exclusion Criteria :

- Patients who are not able to cooperate due to psychotic symptoms at the time of evaluation.
- Those who are not able to give valid informed consent were excluded from the study
- .

3.3.5 Study Groups :

The study population were divided in three groups :

Group 1 : Patients suffering from Schizophrenia for more than 1 year (N=24)., Patients suffering from Other psychotics disorders such as Bipolar Disorder, Major Depressive Disorder for more than 1 year. (N=30).

Group 2 : Healthy controls subjects (N=54)

3.4 Method of collection of data :

A detailed history, assessments of symptoms of psychotic disorder using BPRS Scale, anthropometric examination, physiological examination were performed on all patients who meet the inclusion criteria, both male and female, who were attending psychiatry OPD and admitted to Shri B. M. Patil Medical College, Hospital, and Research Centre Vijayapura, and who have been diagnosed with psychotic disorders such as schizophrenia, bipolar disorders, and major depressive disorders with a duration of more than 6 months

3.4.1. Anthropometrical Data :

Anthropometrical data, viz, Height (feet), Weight (kg), Basal Metabolic Index (BMI) kg/m^2, Waist Circumference (WC) in inches, and physiological data (blood pressure systolic (BPS) and blood pressure diastolic (BPD)) were recorded.

3.4.1.1Height (Feet) : Each subject's height in feet was measured using the Stadiometer while standing without shoes.

3.4.1.2Weight (Kg) : Each individual was weighed using a standard weighing scale with the lightest possible amount of clothes (0.1 kg).

3.4.1.3Waist Cirumceferance : With the help of a standard tailor tape, it was measured with the least amount of clothing at the highest point on the iliac crest and the midway point of the outer border of the ninth costal cartilage (approximately at the level of the umbilicus).

3.4.1.4 Body Mass Index (BMI) Estimation : Weight was recorded to the nearest kilogram (kg) with the subject standing on the weighing machine without shoes and normal clothing. Height was measured with the subject standing upright, barefooted, feet together, back and heels against the upright bar of the height scale, and head upright in a horizontal plane in the "look straight ahead" position. The following formula was used to calculate the body mass index :

BMI = Weight (Kilograms) / Height (meters)^2

3.4.2 Physiological Parameters ; The blood pressure was measured by using a mercurial sphygmomanometer in mmHg.

Sample collection :

Blood samples were collected from all subjects by vein-puncture in different vials, 2 mL in Fluoride Vacutainer, 2ml in a Plain Vacutainer & 1ml of blood will be collected in EDTA Vacutainer as per the parameter to be assayed. Serum was separated within one hour by centrifugation of the blood sample at 3500 rpm for 10 minutes and then the serum was used for the estimation of various parameters and stored at stored in the -80^{0}c deep freezer.

3.4.3 Biochemical Analysis :

For the estimation of blood glucose, random samples of blood were collected in a sodium fluoride vacutainer tube; for the estimation of lipid profile parameters, Random blood samples were taken in a plain vacutainer tube without anticoagulant. And for the molecular

analysis, the samples were collected in EDTA Vacutainer. All the specimens were immediately subjected to assays for Blood glucose, Lipid profile analysis. The tests were carried out on MISAPAUNO Semi autoanalyzer.

3.4.3.1Blood Glucose :

1) Estimation of Blood Glucose :

Method – Estimation done by **Trinder** method

Priniciple :

In the presence of glucose oxidase, the glucose in the sample is oxidized to produce gluconic acid and hydrogen peroxide. The oxidative coupling of 4-aminoantipyrine with phenol, which is catalyzed by the enzyme peroxidase, results in a colored quinoneimine complex, whose absorbance is proportional to the content of glucose in a sample.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + 4\text{AAP} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine Dye} + 2H_2O$$

Composition of Reagent :

Glucose reagent kit was in liquid, ready to-use, single reagent kit which contains:

Reagent 1 : Enzyme reagent

Active Ingredients	Concentration
Glucose oxidase	> 20000U/L
Peroxidase	> 2000U/L
Phenol	10mmol/L
Phosphate Buffer	200mmol/L

Reagent 2 : Glucose Standard : 100mg/dl

The test was programmed and carried out in semi auto analyser – Mispa uno (Agappe)

Assay Parameters :

Reaction Mode	End point
Wavelength – 1	505nm
Wavelength – 2	670nm
Sample Volume (ml	5/10
Reagent Volume (ml	500/1000
Incubation Time	10min.
Reaction Temperature	37^0C
Lag time	NA
Read time	NA
No. of Readings	NA
Reaction Direaction	Inc.
Normal Low	74 mg/dl
Normal High	100 mg/dl
Linearity	500 mg/dl
Standard Conc.	100 mg/dl
Blank with	Reagent
Blank Abs. Limit	0.2
Units	mg/dl

Biological reference interval :

Random Blood Sugar (RBS): 80 -140 mg/ dl (4.44-7.77mmol/l)

Assay Procedure :

Pipette into test tube labelled as	Blank	Standard	Test
Sample	–	–	10µl
Standard	–	10µl	–
Distilled Water	10µl	–	–
Working Reagent	1.0ml	1.0ml	1.0ml

After each addition, thoroughly mix, then incubate for 10 minutes at 370°C. Read the standard absorbance at 505 - 670 nm and test it to the reagent's blank.

Calculation :

$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \text{ X Concentration of Standard (100mg/dl)}$$

3.4.3.2Lipid profile : It contains Serum Total Cholesterol (TC), Serum Triglycerides (TG). Direct High Density Lipoprotein – cholesterol (HDL-C) was done by enzymatic method and also Serum Low Density Lipoprotein Cholesterol (LDL-C) and Very Low Density Lipoprotein Cholesterol (VLDL-C) is calculated by using the Stranded Friedwald's equation - (LDL- C) concentrate = {TC – (VLDL+HDL)} & (VLDL-C) concentrate = TG/5.

2) Estimation of Total Cholesterol :

Method : The **Cholesterol oxidase (CHOD)-peroxidase (POD)** reaction was used to determine the total cholesterol in serum using the enzymatic end-point assay. The test was performed in a semi-automated analyzer using a reagent kit obtained from LiquiCHEK agappe.

Principle :

Cholesterol is determined by the two processes of oxidation and enzymatic hydrolysis. Free cholesterol and fatty acids are released during the enzyme hydrolysis of cholesterol esters by cholesterol esterase. In the subsequent step, cholest–4–en–3–one and hydrogen peroxide are produced from cholesterol by cholesterol oxidase (H_2O_2). By oxidatively combining hydrogen peroxide with 4-aminoantipyrine in the presence of phenol, the peroxidase catalyzes the synthesis of the indicator quinoneimine. The colour quinoneimine absorbs at 510 nm (500–530). The colour is directly and inversely related to the amount of total cholesterol in the sample.

Cholesterol esters $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + Fatty acid

Cholesterol + O2 $\xrightarrow{\text{Cholesterol oxidase}}$ Cholest-4-en -3- one + H_2O

$2H_2O_2$ + 4- Aminoantipyrene + $\xrightarrow{\text{Phenol Peroxidase}}$ Quinoneimine dye +H_2O

Cholesterol Reagents Pack contains :

R 1 Enzymes	Cholesterol Oxidase	>200 U/L
	Cholesterol Esterase	>180 U/L
	Peroxidase	>1000 U/L
	4-Aminoantiyrine	0.5mmol/L
	Phenol	24mmol/L
Cholesterol Standard Conc.		200mg/dl

Automated parameters :

Mode of Reaction	**End point**
Wavelength 1	505 (492-550)nm
Wavelength 2	630nm
Slope of Reaction	Increasing
Temperature	37^0C
Standard Concentration	200mg/dl
Blank	Reagent
Linearity	600mg/dl
Incubation time	5min.
Sample Volume	10ml
Reagent Volume	1000ml
Cuvette	1 cm light path

Biological reference interval :

Total Cholesterol (TC) : 150 -220 mg/ dl

Assay Procedure :

Pipette into test tube labelled as	**Blank**	**Standard**	**Test**
Sample	–	–	10µl
Standard	–	10µl	–
Distilled Water	10µl	–	–
Working Reagent	1.0ml	1.0ml	1.0ml

After each addition, thoroughly mix, then incubate for 5 minutes at 37°C. Read the standard absorbance at 505 - 630 nm and test it to the reagent's blank.

Calculation :

$$\text{Cholesterol Conc. (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \text{ X } 200$$

3) Estimation of Triglycerides :

Method : The Glycerol Phosphate Oxidase (GPO) reaction was used to determine the serum triglycerides in serum using the enzymatic end-point assay. The test was performed in a semi-automated analyzer using a reagent kit obtained from LiquiCHEK agappe.

Principle :

Triglycerides are converted into free fatty acids and glycerol by lipoprotein lipase. Adenosine triphosphate (ATP) is then used by glycerol kinase to phosphorylate the glycerol, creating glycerol 3-phosphate and adenosine diphosphate (ADP). Dihydroxy- acetone phosphate is formed when glycerol-3-phosphate is treated with glycerol phosphate oxidase (GPO), resulting in the production of hydrogen peroxide (H_2O_2). Quinoneimine dye is produced via, the reaction of 4-aminoantipyrine (4-AAP) and 4-chlorophenol (4-CP) with H_2O_2.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipoprotein Lipase}} \text{Glycerol} + \text{Fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycrol-5-phosphates} + \text{ADP}$$

$$\text{Glycrol-5-phosphates} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate} + H_2O_2.$$

$$2H_2O_2. + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{POD}} \text{Violet coloured complex}$$

Cholesterol Reagents Pack contains :

Triglyceride reagent kit was in liquid, ready to use, single reagent kit which contains:

R 1 Enzymes	Lipoprotein lipase	>1800 U/L
	Glycerol kinase	>450 U/L
	Glycerol-3-phoshate Oxidase	>3500 U/L
	4-Aminoantiyrine	0.9 mmol/L
	Peroxidase	>0.4 mmol/L
	ATP	3.15 mmol/L
Triglycerides Standard Conc.		200 mg/dl

Automated parameters :

Mode of Reaction	End point
Wavelength 1	546 (540-560)nm
Wavelength 2	630 nm
Slope of Reaction	Increasing
Temperature	37^0C
Standard Concentration	200mg/dl
Blank	Reagent
Linearity	1000mg/dl
Incubation time	5min.
Sample Volume	10ml
Reagent Volume	1000ml
Cuvette	1 cm light path

Biological reference interval :

Serum Triglycerides (TG) : Male : 60-165 mg/ dl, Female : 40-140 mg/dl

Assay Procedure :

Pipette into test tube labelled as	Blank	Standard	Test
Sample	–	–	10µl
Standard	–	10µl	–
Distilled Water	10µl	–	–
Working Reagent	1.0ml	1.0ml	1.0ml

After each addition, thoroughly mix, then incubate for 5 minutes at 37°C. Read the standard absorbance at 540 - 630 nm and test it to the reagent's blank.

Calculation :

$$\text{Serum Triglycerides Conc (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times 200$$

3) Estimation of Direct High Density lipoprotein – Cholesterol :

Methods : Selective Inhibition Method.

Principle : The electrostatic interaction between polyanion and cationic compounds inhibits the reaction between cholesterol other than HDL and the enzyme for cholesterol testing. The

free cholesterol in HDL is converted to hydrogen peroxide by cholesterol oxidase. The oxidative condensation of EMSE and 4-AA is produced by hydrogen peroxide and peroxidase, and the absorbance of the resulting red-purple quinone is tested to determine HDL cholesterol levels..

$$\text{HDL (cholesterol esters)} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{HDL (free cholesterol)} + \text{Free fatty acids}$$

$$\text{HDL (free cholesterol)} + O2 + H^+ \xrightarrow{\text{cholesterol oxidase}} \text{Cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{4-AA} + \text{EMSE} + H_3 + O \xrightarrow{\text{Peroxidase}} \text{Violet quinone} + 5\ H_2O$$

Reagent composition :

The R1 & R2 reagent kit was in liquid, ready to use.

Reagent/ Component	Description
HDL–C Direct R1	N—ethyl-N-(3- methylphenyl) - N'succinylethyenediame (EMSE)
HDL–C Direct R2	Cholesterol Oxidase 4 Aminoantipyrin (4-AA)
HDL–C Direct calibrator	

Automated parameters :

Mode of Reaction	End point
Wavelength 1	578nm(578-610nm)
Wavelength 2	630nm(630-700nm)
Slope of Reaction	Increasing
Temperature	37^0C
Standard Concentration	100mg/dl
Blank	Reagent
Linearity	150mg/dl
Incubation time	5+5min.
Sample Volume	3µl
Reagent1 Volume	450 µl
Reagent2 Volume	150 µl
Cuvette	1cm light path

Biological reference interval :

Serum (Direct HDL-C) : Male : 35-80 mg/ dl, Female : 42-88 mg/dl

Assay Procedure :

Pipette into test tube labelled as	Blank	Standard	Test
Sample	–	–	5 µL
Standard	–	5 µL	–
Distilled Water	5 µL	–	–
Working Reagent 1	450 µL	450 µL	450 µL
Mix & incubate for 5 min at 37^0C			
Reagent 2	150 µL	150 µL	150 µL
Mix and incubate for 5 min at 37oC and read absorbance of calibrator & sample against reagent blank at 578 & 630 nm.			

Calculation :

$$\text{Direct HDL-C Conc (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times 100$$

4) Estimation of LDL Cholesterol :

Calculation as per FRIEDEWALD'S Formula :

LDL = Total Cholesterol [HDL + (Triglyceride / 5)]

Biological reference interval : Normal Serum LDL level range: 30-100 mg/dl.

5) Estimation of VLDL Cholesterol :

It is calculated by the formula **-** Triglycerides/5

Biological reference interval : Normal Serum VLDL range: 10-30 mg /dl.

3.4.4. Expression analysis :

Gene expression studies

Expression analysis of RELN genes was performed by Real-time Polymerase Chain Reaction (RT-PCR/qPCR).

a) RNA Extraction from TRIZOL (Sigma Aldrich Catalog Number T9424)

Method :

The TRIZOL reagent (Sigma-Aldrich) was used in a single-step RNA isolation procedure to separate total RNA from frozen EDTA blood samples of drug-treated and healthy control groups (Chomczynski and Sacchi, 1987).

Reagent required for RNA isolation :

I. Chloroform (100ml)
II. Isopropanol (100ml)
III. 75% Ethanol
IV. 1mM Sodium Phosphate
V. 4N acetic acid

Step 1. Sample Preparation :

750 µl of TRIzol LS Reagent were added to 250 µl blood sample (no more than 5 x 106 cells). Transferred to the 2ml microtubes.

Phase Separation :

The samples were incubated for 5 mins at room temperature then added 200 µl of chloroform per ml of TRI Reagent used. Covered the sample tightly, mixed vigorously for 15 seconds, and kept for incubation for 2–15 minutes at room temperature. Then centrifuged the mixture at 12,000 x g for 15 minutes at 2-8^{0}C. After the centrifugation we got 3 phases: a red organic phase (containing protein), an interphase (containing DNA), and a colourless upper aqueous phase (containing RNA)..

Step 2. RNA Isolation :

RNA Precipitation :

Then transferred the aqueous phase to 2ml micro tubes and then added 500 µl of 2-propanol per ml of TRI Reagent used for Sample Preparation, step 1 and mix. Samples were kept for incubation 5–10 minutes at room temperature. Centrifuged at 12,000 x g for10 minutes at 2-8^{0}C. Then we got RNA precipitate in the form a pellet on the side and bottom of the tube.

RNA Wash :

Removed the supernatant and washed the RNA pellet by adding a minimun of 1 ml of 75% ethanol Vortexed the samples and then centrifuged at 7,500 x g for 5 minutes at 2-8 0 c.

Step 3. RNA Solubilisation :

Dried the RNA pellet for 5–10 minutes by air- drying. Added appropriate volume of water, to the RNA pellet. Mixed by repeated pipetting with a micropipette at 55–60 0 c for 10–15 minutes**c**

b) Quantification of RNA by Multi mode Reader (Teckon) :

We used the Multimode reader (Teckon) for the quantification of RNA. Multimode Reader is a micro-volume UV spectrophotometer specifically designed for the measurement of nucleic acids and purified proteins. Its unique technology holds 0.5-2.5 ul samples between

upper and lower measurement surfaces without the use of a cuvette. It measures the samples in less than 2 seconds with a high degree of accuracy and reproducibility. The Multimode reader works on the principle, "Nucleic acids absorb light at a wavelength of 260/280 nm and when 260 nm light source shines on a sample, the amount of light that passes through the sample can be measured, and the amount of light absorbed by the sample can be inferred. For single stranded RNA, an Optical Density (OD) of 1 at 260 nm correlates to a RNA concentration of 50 ng/µl, so that RNA concentration can be easily calculated from OD measurements."

c) Primer Designing for RELN Gene :

Web-based, freely available program PRIMER 3, which is widely accepted, is used for designing PCR primers; primer 3 is a bioinformatics tool that helps in designing the primers for the target region in the given nucleotide sequence as per the requirement of the user or applications. Screening of the donors for the RELN primer sequence were performed by using 5' CATGGTTGCAAGTGTGACCC-3' and 5'-AAACCAGGGCCTTACCACTG -3' The primers of ACTB were 5'-AAGTCCCTCACCCTCCCAAAAG-3'and 5' AAGCAATGCTGTCACCTTCCC -3'

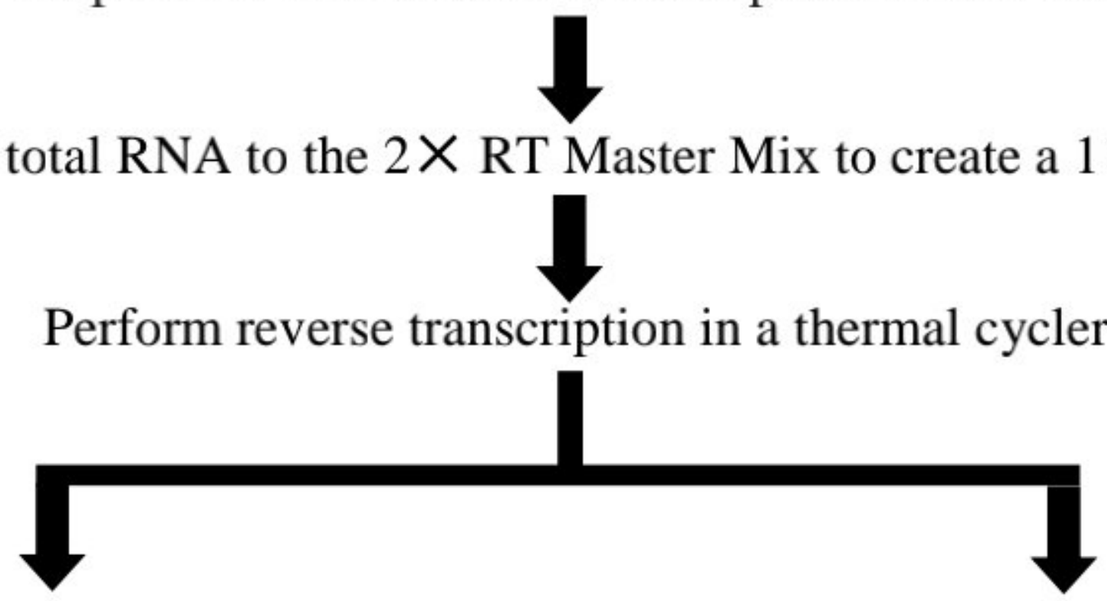
d) Agarose Gel Electrophoresis of RNA

Gel electrophoresis is one of the molecular biology techniques used toseparate RNA depending on the length of fragments. It is a widely used and accepted method, to estimate the size of RNA fragments or to separate proteins by charge. Nucleic acid molecules are separated based on an electric field to move the negatively charged molecules through an agarose matrix. Shorter molecules move faster and migrate farther than longer ones because shorter molecules migrate more easily through the pores of the gel. This phenomenon is called sieving.

e) cDNA synthesis :

Using a High Capacity cDNA Reverse Transcription Kit from Thermo Fisher and following the manufacturer's instructions, a single standard cDNA was produced from whole RNA.

Overview : Using the High-Capacity cDNA Reverse Transcription Kits, single-stranded cDNA may be produced from total RNA.



2× RT master mix were prepared by using the kit for 20 µl reactions. RT PCR Master mix prepared in ice by adding as follows :

Components	**Volume/ Reaction (ml)**
10× RT Buffer	2.0µl
25× dNTP Mix (100 mM)	0.8µl
10× RT Random Primers	2.0µl
MultiScribe™ Reverse Transcriptase	1.0µl
RNase Inhibitor	1.0µl
Nuclease-free H_2O	3.2µl

Preparation of cDNA RT reactions :

Taken 10 µL of 2× RT master mix into each on individual tubes. Pipette out 10 µL of RNA sample into each well, pipetting up and down two times to mix. Closed the tubes. Briefly centrifuged the tubes to spin down the contents and to eliminate any air bubbles. Placed the tubes on thermal cycler. Program the thermal cycler conditions using one of the thermal cycler –

Step 1 - 25^0C for 10 mint.

Step 2 - 37^0C for 120 mint.

Step 3 - 85^0C for 5mint. and 4^0Cfor ∞

Total reaction volume was to 20 µL. Loaded the reactions on to the thermal cycler. Started the reverse transcription run.

f) Quantitative Real-time (RT-PCR) analysis :

Real-time RT-PCR for RELN gene in mRNA was carried out by using SYBR1 Green QPCR Master Mix, 2 µl of cDNA amplified by PCR in 25 µL reactions containing 12.5 µl of SYBR green reagents and 0.2 mM of each of the primers. The initial incubation for 10 min at 95˚C will be followed by 40 cycles at 95˚C for 15 s and 60 0 for 1 min. The expression level will be determined using 2-ΔΔCt and normalized to βactin and represented as fold change. (The primers of RELN gene for RT-PCR were 5' CATGGTTGCAAGTGTGACCC-3' and 5'-AAACCAGGGCCTTACCACTG -3'; the primers of βactin for RT-PCR were 5'-AAGTCCCTCACCCTCCCAAAAG-3'and 5' AAGCAATGCTGTCACCTTCCC -3'.)

3.5 Statistical Analysis

1. The data obtained is entered in a Microsoft Excel sheet, and statistical analyses are performed using a statistical package for the social sciences (SPSS) (Version 20).
2. Results are presented as Mean, SD, counts and percentages, and diagrams.
3. Normally distributed continuous variables between two groups will be compared using an Independent t-test and ANOVA test for not normally distributed variables. Mann Whitney U test and Kruskal-Wali H test were used. Categorical variables between the two groups were compared using the Chi-square test.
4. For Normally distributed continuous variables to see the correlation between two variables, we used Pearson Correlation coefficient value, the Spearman's rho Correlation coefficient value is used.
5. $p<0.05$ will be considered statistically significant. All statistical tests will perform two-tailed

Results

Chapter - V

4 Results :

The present analytical cross – sectional study was carried out in Dept. of Biochemistry in collaboration with Dept. of Psychiatry and Centre For Advanced Medical Research (CAMER) of BLDE (DU's). Shri. B. M. Patil Medical College, Hospital and Research centre, Vijayapura.

Duration of the study – Samples were collected from July 2022 to Sept.2022.

Before the start of the study intuitional ethical clearance certificate and informed consent was obtained from the participant. The utmost care about confidentiality of the patient's data was taken according to Helsinki Declaration.

Total samples collected were 108. These were divided in two groups

Group 1 – Study group (54 participants) – here 24 cases of SCZ + 12 cases of BPAD + cases 15 of MDD + 03 cases of PSY.

Group 2 Control group – 54 age and sex matched healthy individiuals.

Table No. 3 No. of Subjects in study group and control group.

Gender	CASES		CONTROL	
	No. of Patients	Percentage	No. of Patients	Percentage
Female	29	53.7	28	50.0
Male	25	46.3	28	50.0
Total	54	100.0	56	100.0

In the study group the patients showed positive and negative symptoms as mentioned in the material and methodology. The data obtained was statistically analyzed and presented as tables and figures.

The anthropometric measurements, which comprised Age (in years), Height (in centimeters), Weight (in kilograms), Body Mass Index (in kilograms/meters2), and physiological parameters including blood pressure, were recorded in the control and study groups. There were 54 total participants in the control group. In the study group, there were a total of 54 participants.

Clinical and Demographic profile of all the subjects :

Table 4 and Figure 3 shows Mean and SD, and level of significance of each parameter in the study group as compared to control group. Both study group showed the similar age group. There was no significant difference in the systolic and diastolic blood pressure in the study group as compared to control group. There was highly significant increase ($p<0.001$) in BMI and WC in the study as compared to control group.

Table No. 4 Clinical and Demographic profile of all the subjects

Parameters	Case Group (N= 54)	Control Group (N=54)	p-Value
Age (Years)	40.83±9.931	44.46±10.914	0.071
Weight (kg)	59.65±17.13	58.03±10.41	0.428
Height (cm)	148.81±6.83	155.61±8.85	<0.001***
BMI (kg/m^2)	27.45±5.60	24.03±3.60	<0.001***
WC (cm)	81.93±13.10	60.92±9.34	<0.001***
Systolic BP	123.02±16.22	124.29±9.88	0.578
Diastolic BP	83.61±9.394	81.79±9.167	0.387

***p<0.001 Very Highly Significant.

BMI - Body Mass Index, WC – Waist Circumference

Figure No. 3 : Graph showing Clinical and Demographic profile of all the subjects

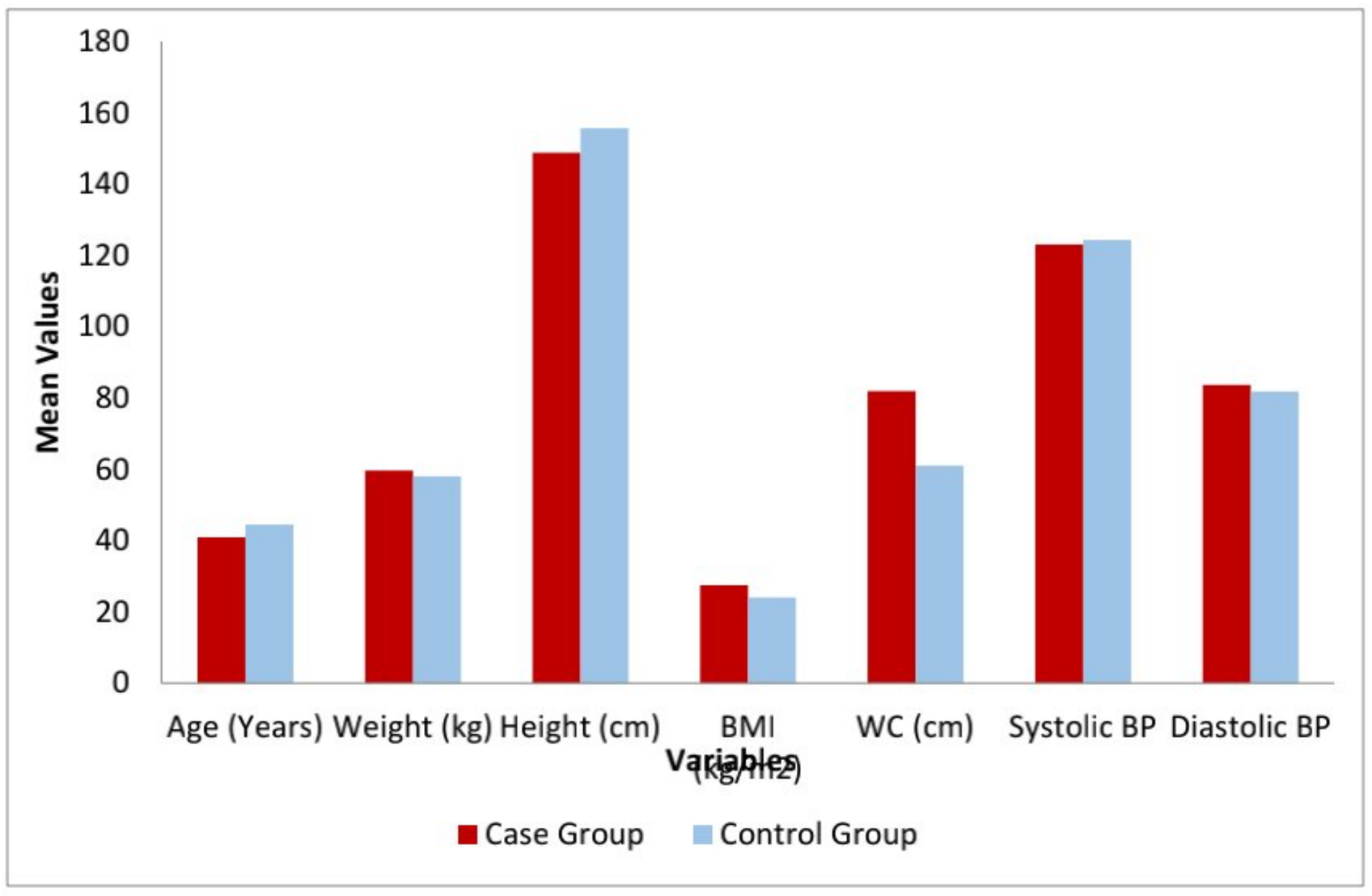
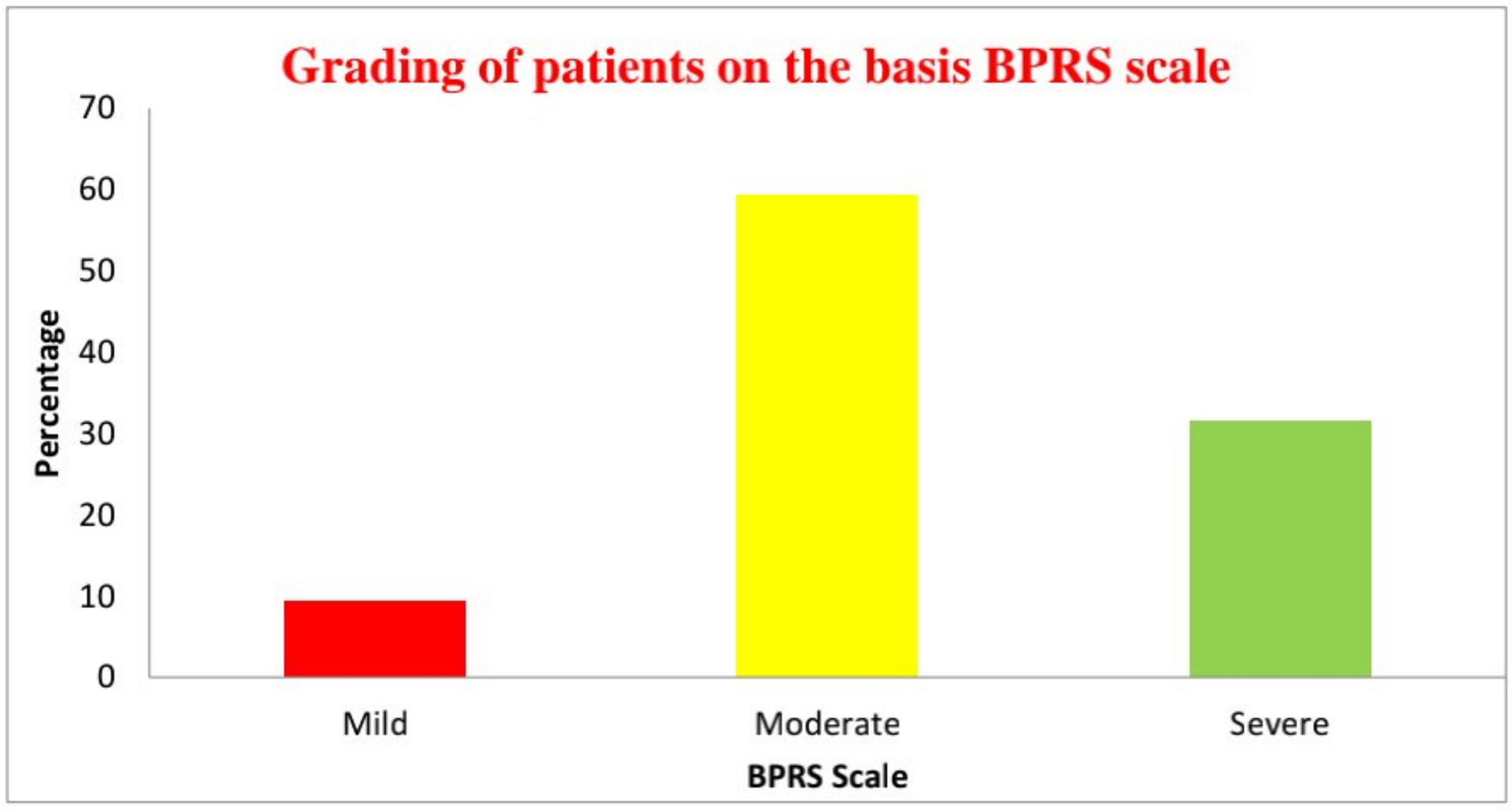


Figure 4 show that the maximum no of participants showed moderate score on the basis of BPRS Scale.

Figure No. 4 : Graph showing grading of patients on the basis BPRS scale



The study group participants were further divided on the basis of BMI as underweight (3.70%), Normal (27.77%), Overweight (42.59%) and Obese (25.92%). (Table No. 5)

Table No. 5 Distribution of study group on the basis of BMI

BMI Group	Study Group	No. of cases	Percentages
Underweight	17.5±0.707	2	3.70%
Normal	22.64±1.717	15	27.77%
Overweight	27.11±1.714	23	42.59%
Obese	33.62±5.158	14	25.92%

The data in table 6 reflects the highly significant increase in RBS in the study group as compared to control group ($p<0.001$). Similarly, the lipid indices like serum Total cholesterol, LDL-C were highly significantly increased in study as compared to control group. ($p<0.001$). Whereas HDL-C showed significant decrease ($p<0.001$) in study group. Triglycerides levels in were increased in study but it was not statistically significant. The significantly higher lipid ratios in the study group indicates that the patients suffering from all psychotics disorders are more prone to future metabolic syndrome as well as cardiovascular diseases.

Table No. 6 Mean ± SD of biochemical parameters in study group and control group

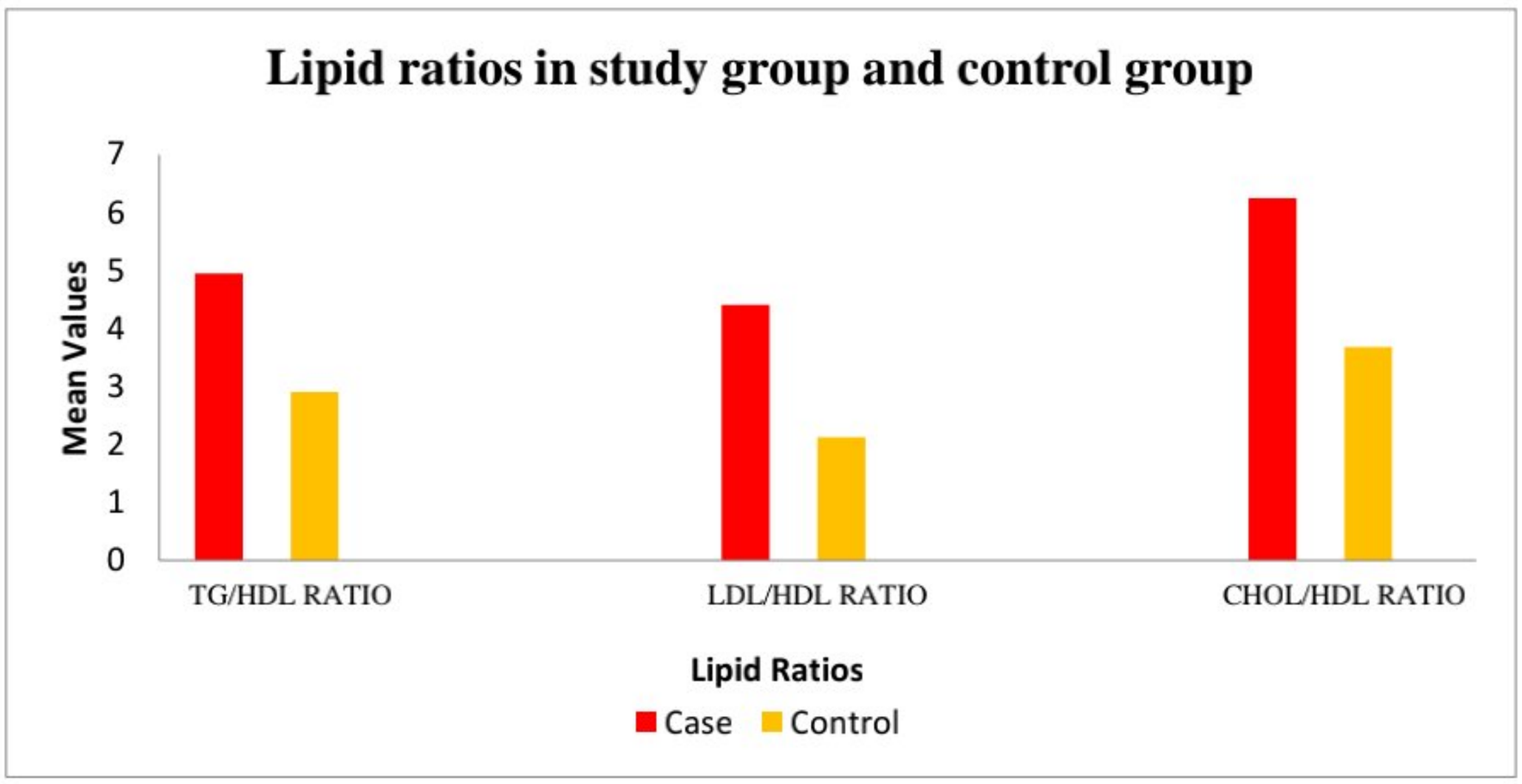
Parameters	Case Group (N= 54)	Control Group (N=54)	p-Value
RBS (mg/dl)	147.02±37.368	123.59±18.278	0.001***
TC (mg/dl)	223.11±46.683	167.23±16.739	0.001***
Serum TG (mg/dl)	155.94±66.873	131.54±20.732	0.312
Serum Direct HDL - C	25.85±7.181	45.88±6.284	0.001***
Serum LDL- C	167.41±48.919	95.79±13.309	0.001***
Serum VLDL- C	31.81±13.461	26.77±5.281	0.239
TG/HDL Ratio	4.9475±2.46939	2.9046±0.53115	0.001***
LDL/HDL Ratio	4.4102±2.24474	2.1284±0.40831	0.001***
CHOL/HDL Ratio	6.2504±2.79128	3.6772±0.44596	0.001***

Values are shown as the Mean±SD. **RBS :** Random Blood Sugar, **Serum TC :** Total cholesterol, **Serum TG :** Serum Triglycerides, **Serum Direct HDL – C :** Serum Direct High Density Lipoprotein – Cholesterol, **Serum LDL – C :** Serum Low Density lipoprotein- Cholesterol, **Serum VLDL – C :** Serum Very Low Density lipoprotein- Cholesterol.

***p<0.001

Lipid profile Ratios :

Fig No. 5 Graph showing Lipid ratios in study group and control group



When the data was analysed with IDF and NCETP – ATP- III criteria is found that 54.5% female and 45.5% males were found to be prone for developing Mets. When the underlying cause for developing for metabolic syndrome was analysed it was observed that the use of

antipsychotics drugs like Clozapine 43.2%, Olanzapine 111.7%, Risperidone 18.3%, and Quetiapine 18.3%, was observed in the study group.(Fig.5)

Fig. No 6 : Graph showing a various antipsychotics drugs

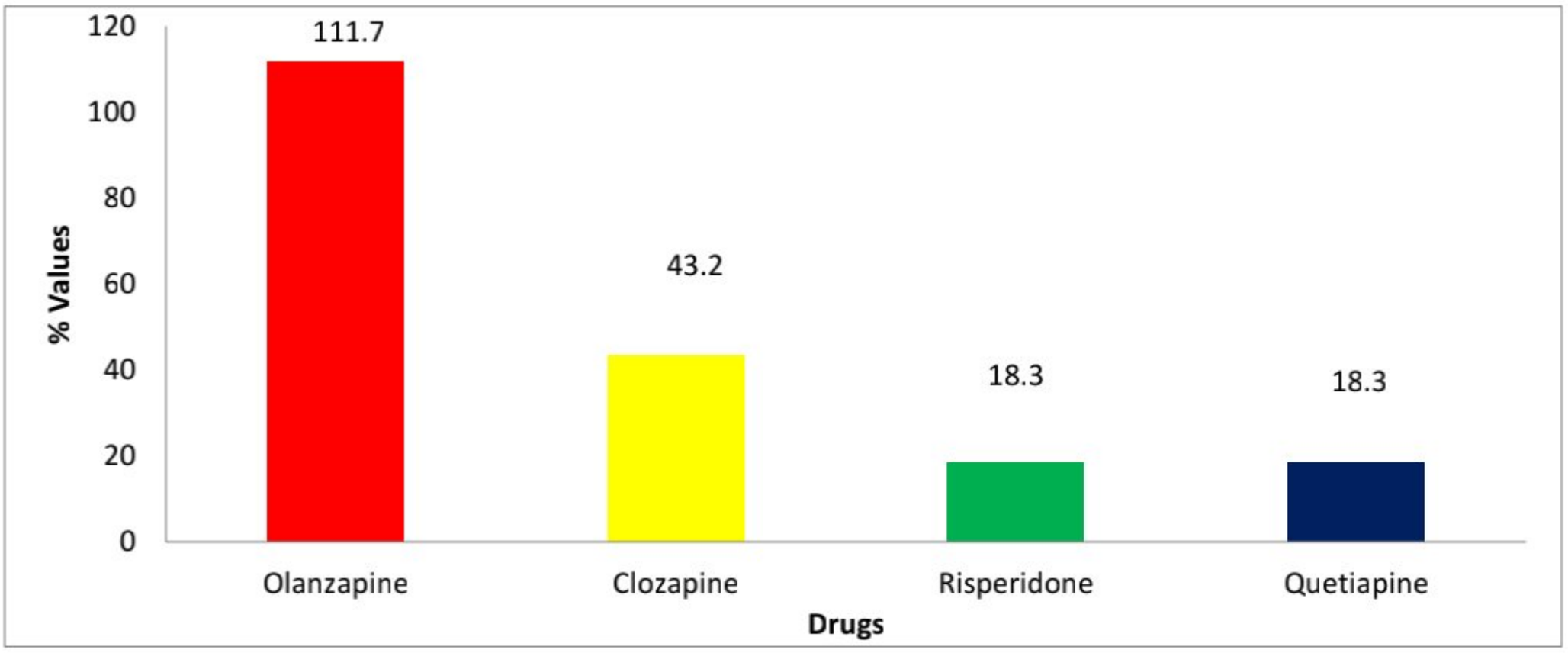


Table No. 7 shows the data of SCZ participants. The biochemical parameters in comparison with control group show significant increasing RBS, TC, TG, LDL- C, VLDL- C and lipid ratios TG/HDL, LDL/HDL, CHOL/HDL and serum HDL-C significantly decreased.

Table No. 7 Mean±SD of SCZ group and control group

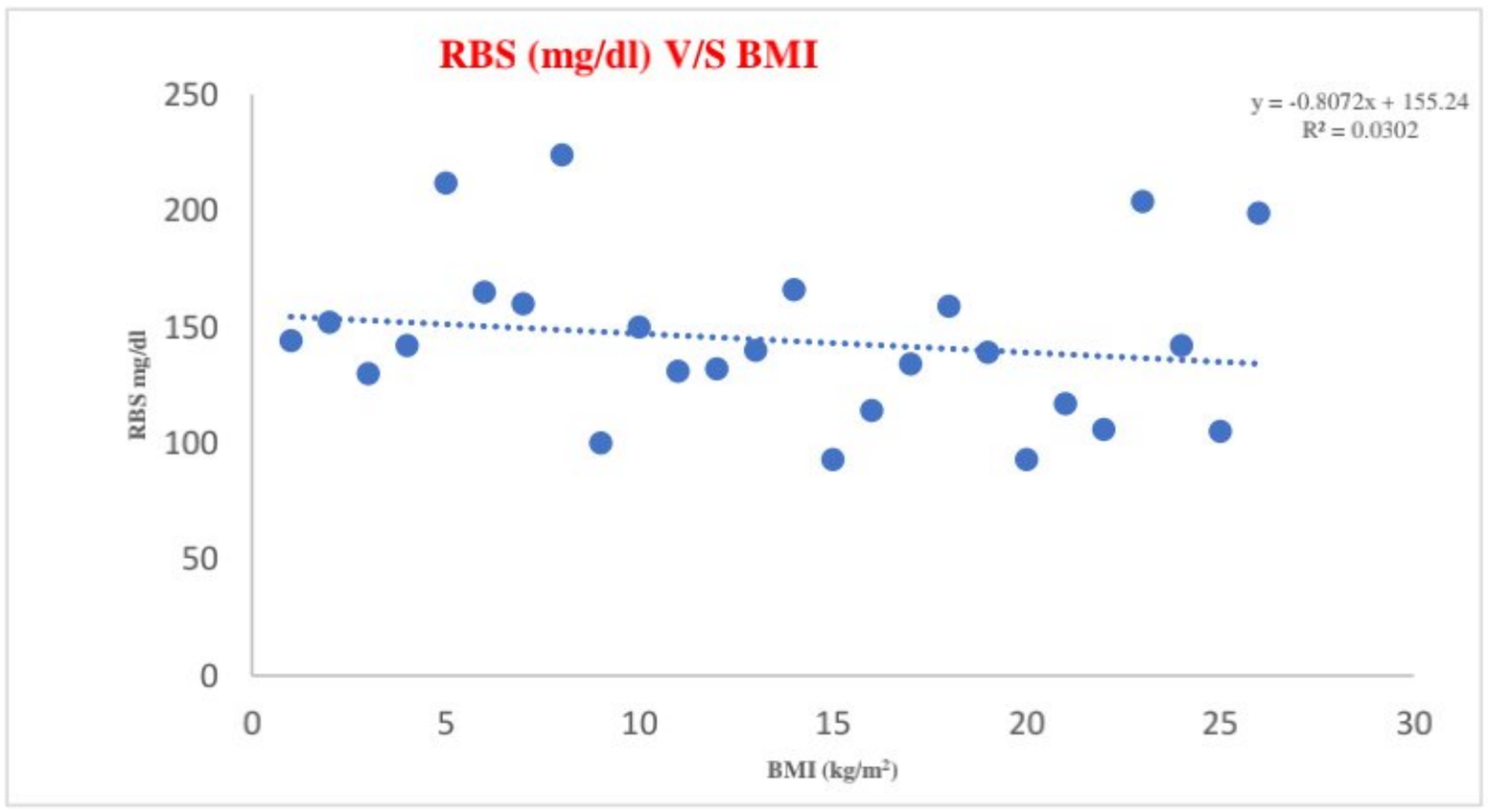
Parameters	SCZ Group (N= 24)	Control Group (N=54)
RBS (mg/dl)	149.4±40.6	123.59±18.278
TC (mg/dl)	213±43.5	167.23±16.739
Serum TG (mg/dl)	167.7±80.7	131.54±20.732
Serum Direct HDL - C	26.4±6.18	45.88±6.284
Serum LDL- C	157.1±49.9	95.79±13.309
Serum VLDL- C	33.47±16.14	26.77±5.281
TG/HDL Ratio	5.09±2.77	2.9046±0.53115
LDL/HDL Ratio	4.58±2.49	2.1284±0.40831
CHOL/HDL Ratio	6.76±3.07	3.6772±0.44596

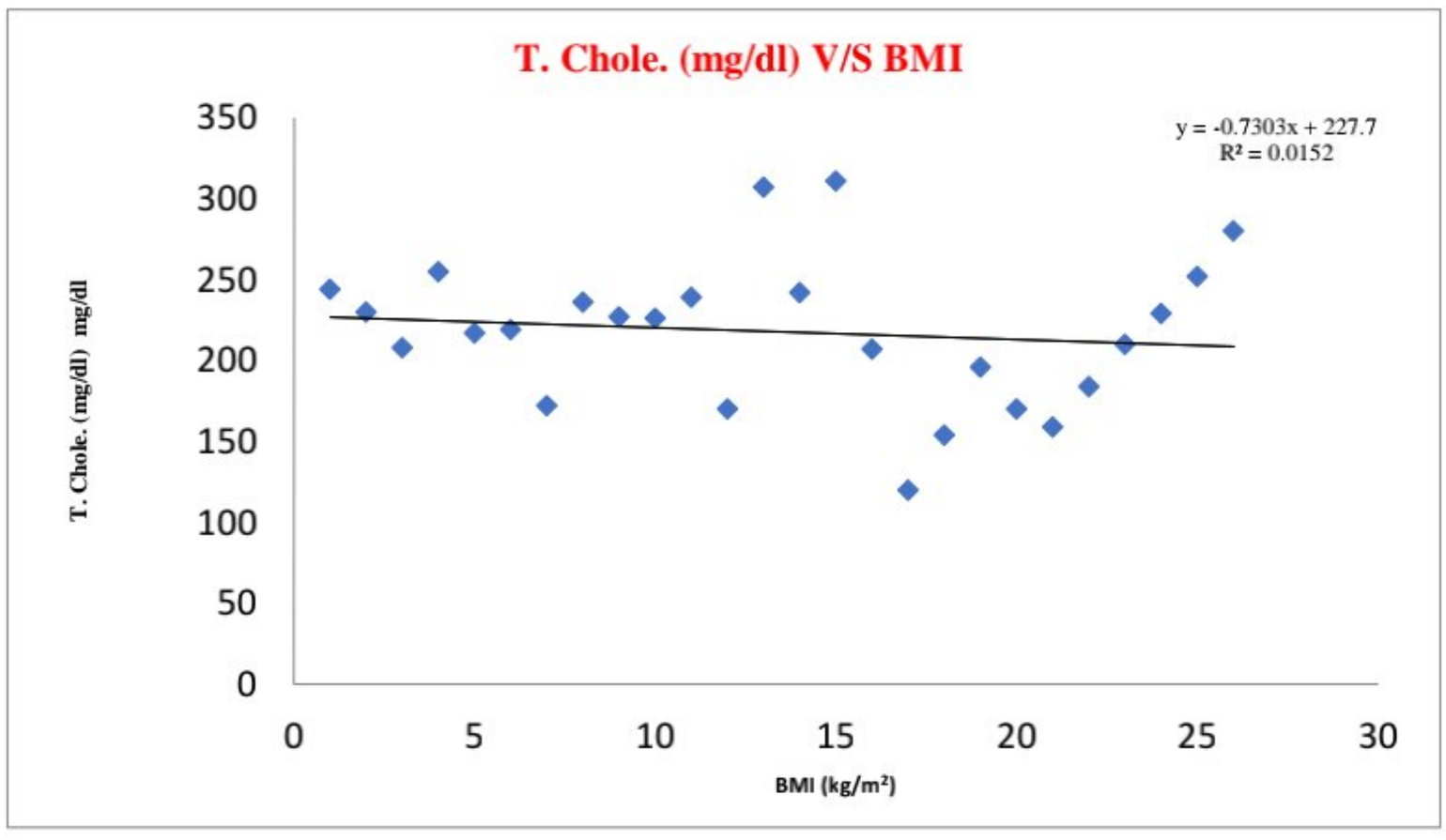
Table No. 8 shows a BMI and biochemical parameters such as blood glucose and lipid profile levels had a significant positive correlation, whereas lipid ratios had a negative correlation with BMI (BMI v/s TG/HDL r : -0.090, p : 0.515. BMI v/s LDL/HDL r : -0.092, p : 0.507. BMI v/s CHOL/HDL r : -0.054, p : 0.701).

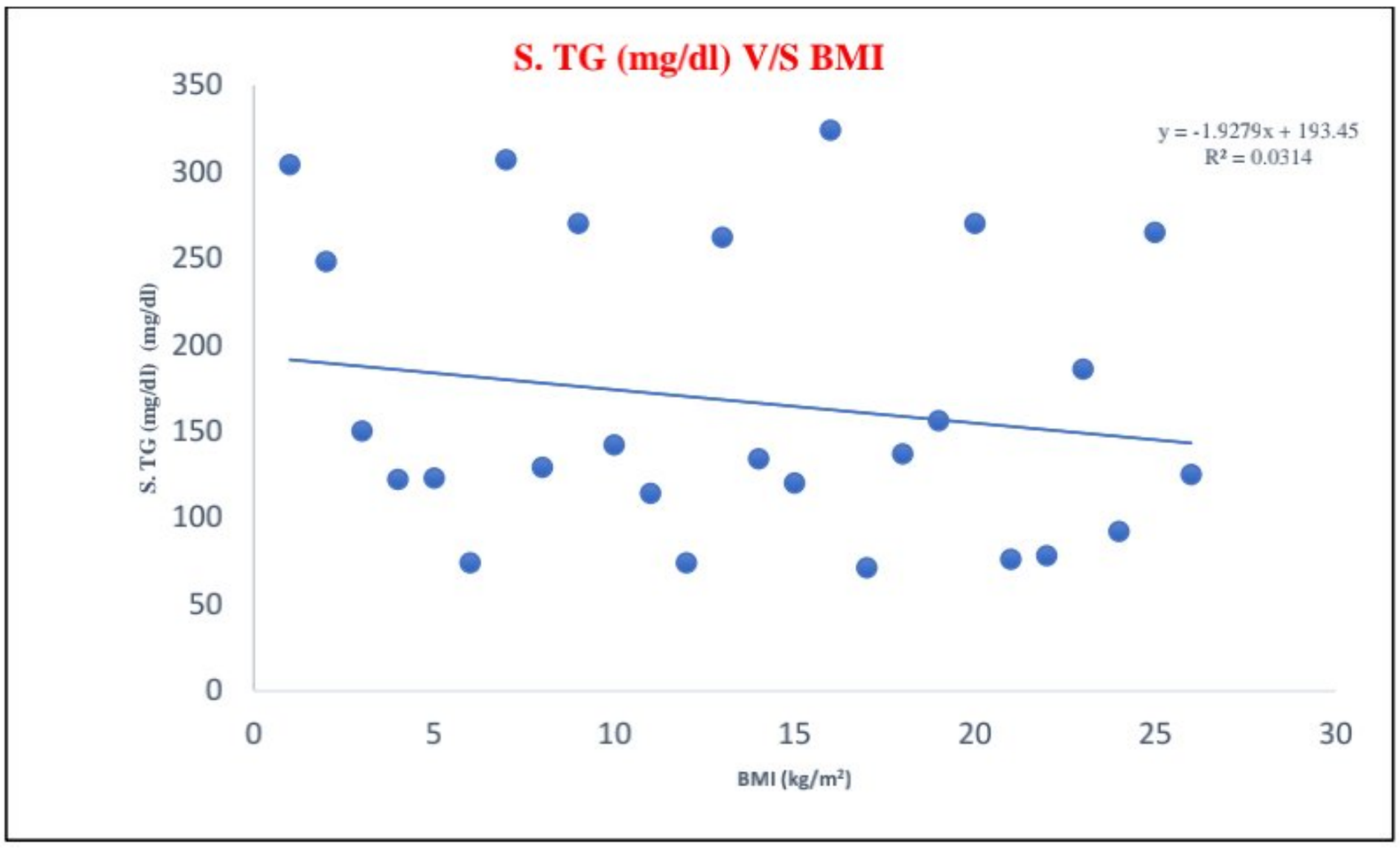
Table No 8 - Bivariate correlation between BMI and Biochemical parameters in SCZ group

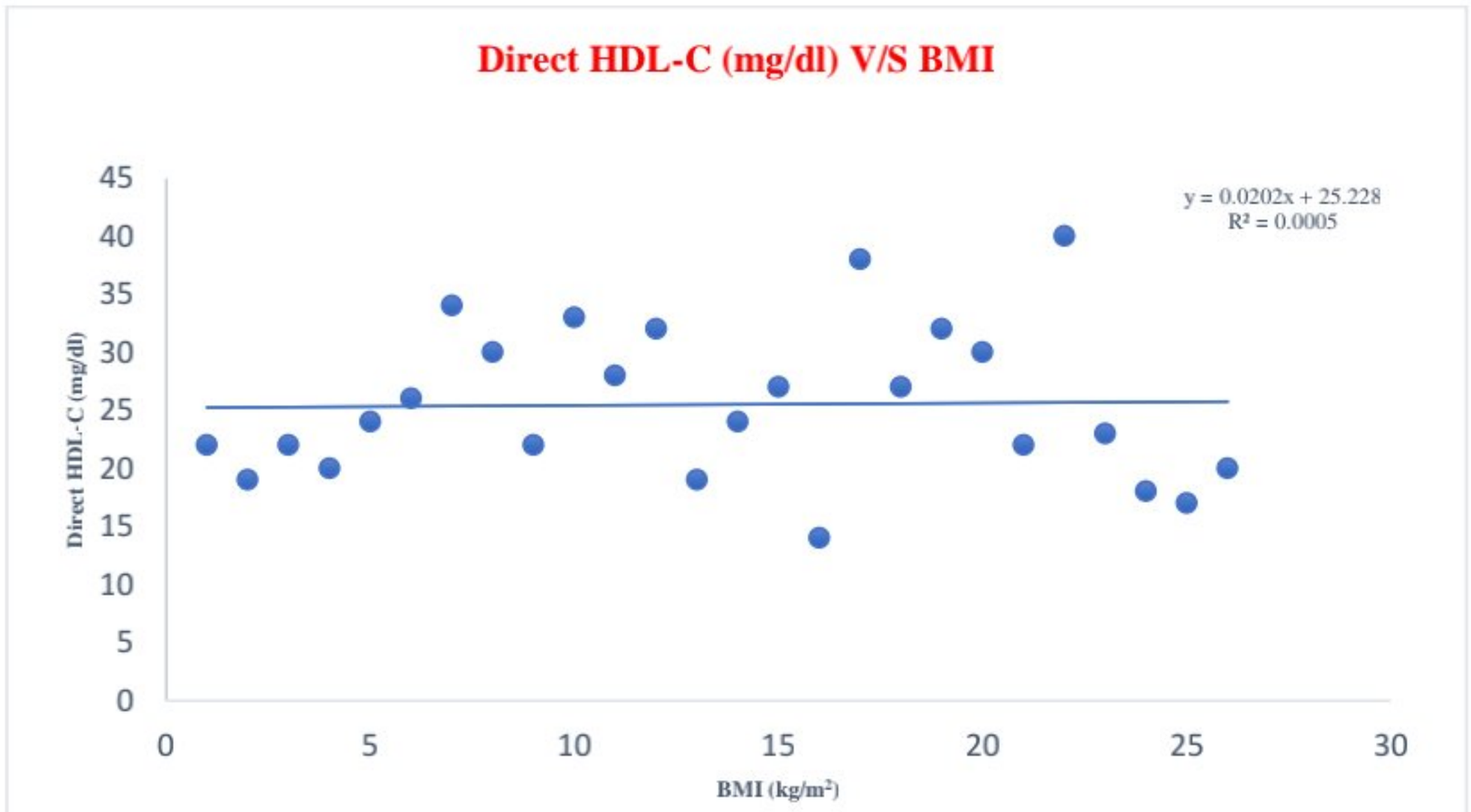
Biochemical parameters	BMI	
	r- value	p- value
RBS (mg/dl)	-0.011	0.957
Serum TC (mg/dl)	0.112	0.587
Serum TG (mg/dl)	0.010	0.960
Direct HDL-C (mg/dl)	0.096	0.642
Serum LDL-C (mg/dl)	0.128	0.534
Serum VLDL-C (mg/dl)	0.003	0.990
BMI v/s TG/HDL	-0.090	0.515
BMI v/s LDL/HDL	-0.092	0.507
BMI v/s CHOL/HDL	-0.054	0.701

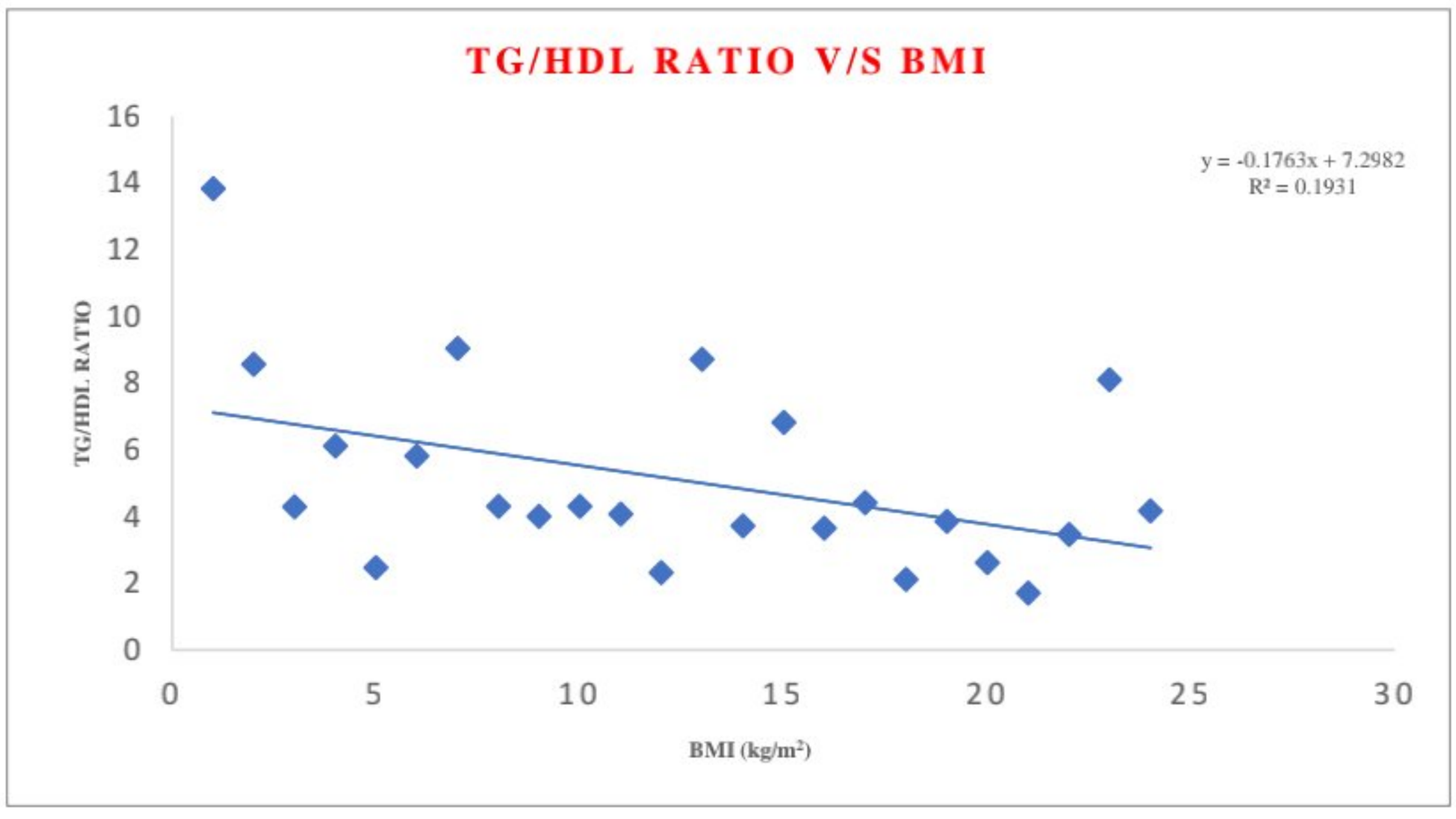
Fig. No. 7 Graph Showing Bivariate correlation between BMI and Biochemical parameters in SCZ group

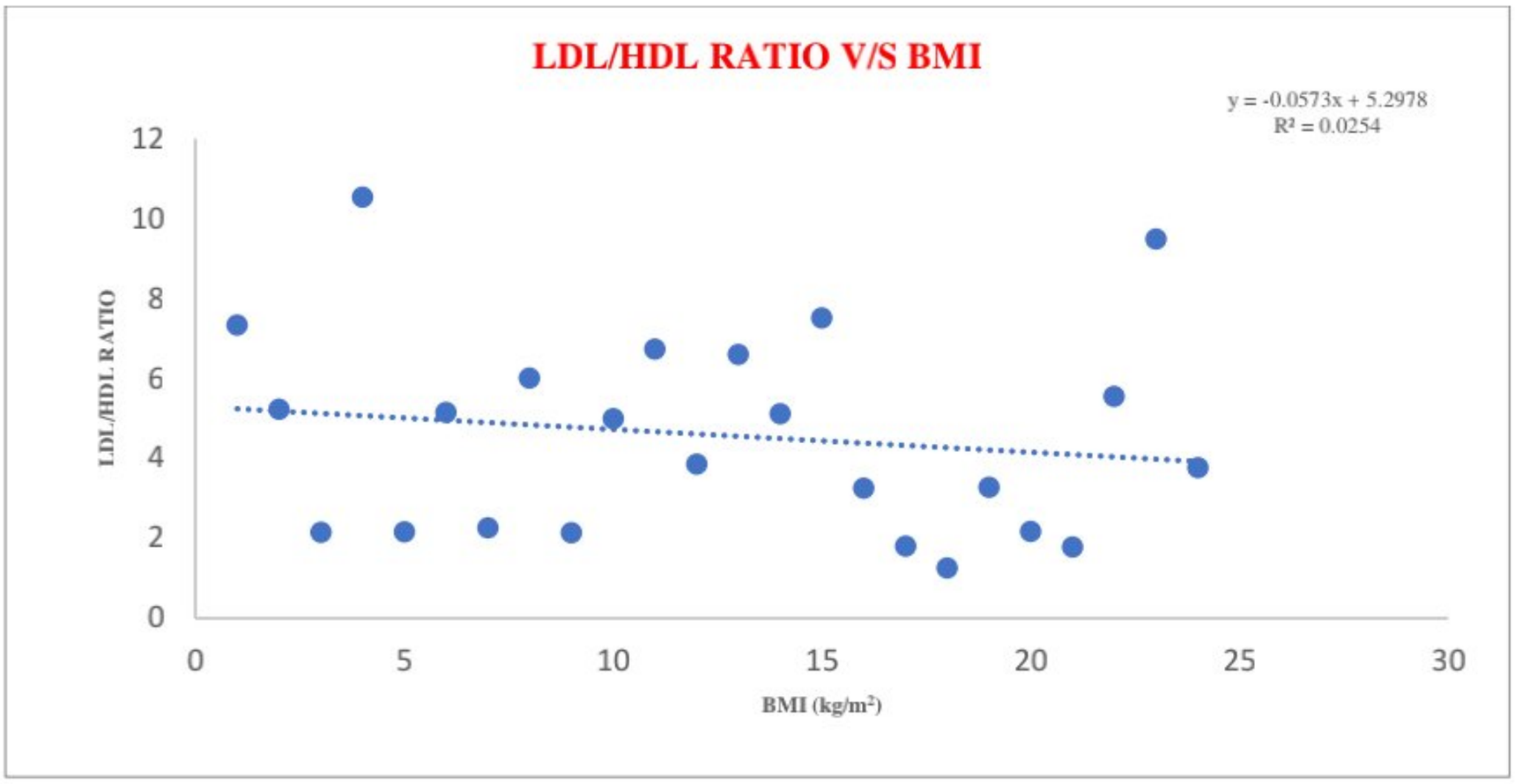





S. TG (mg/dl) V/S BMI
y = -1.9279x + 193.45
R² = 0.0314
S. TG (mg/dl) (mg/dl)
350
300
250
200
150
100
50
0
0
5
10
15
20
25
30
BMI (kg/m²)


Direct HDL-C (mg/dl) V/S BMI
y = 0.0202x + 25.228
R² = 0.0005
Direct HDL-C (mg/dl)
45
40
35
30
25
20
15
10
5
0
0
5
10
15
20
25
30
BMI (kg/m²)


TG/HDL RATIO V/S BMI
y = -0.1763x + 7.2982
R² = 0.1931
TG/HDL RATIO
16
14
12
10
8
6
4
2
0
0
5
10
15
20
25
30
BMI (kg/m²)



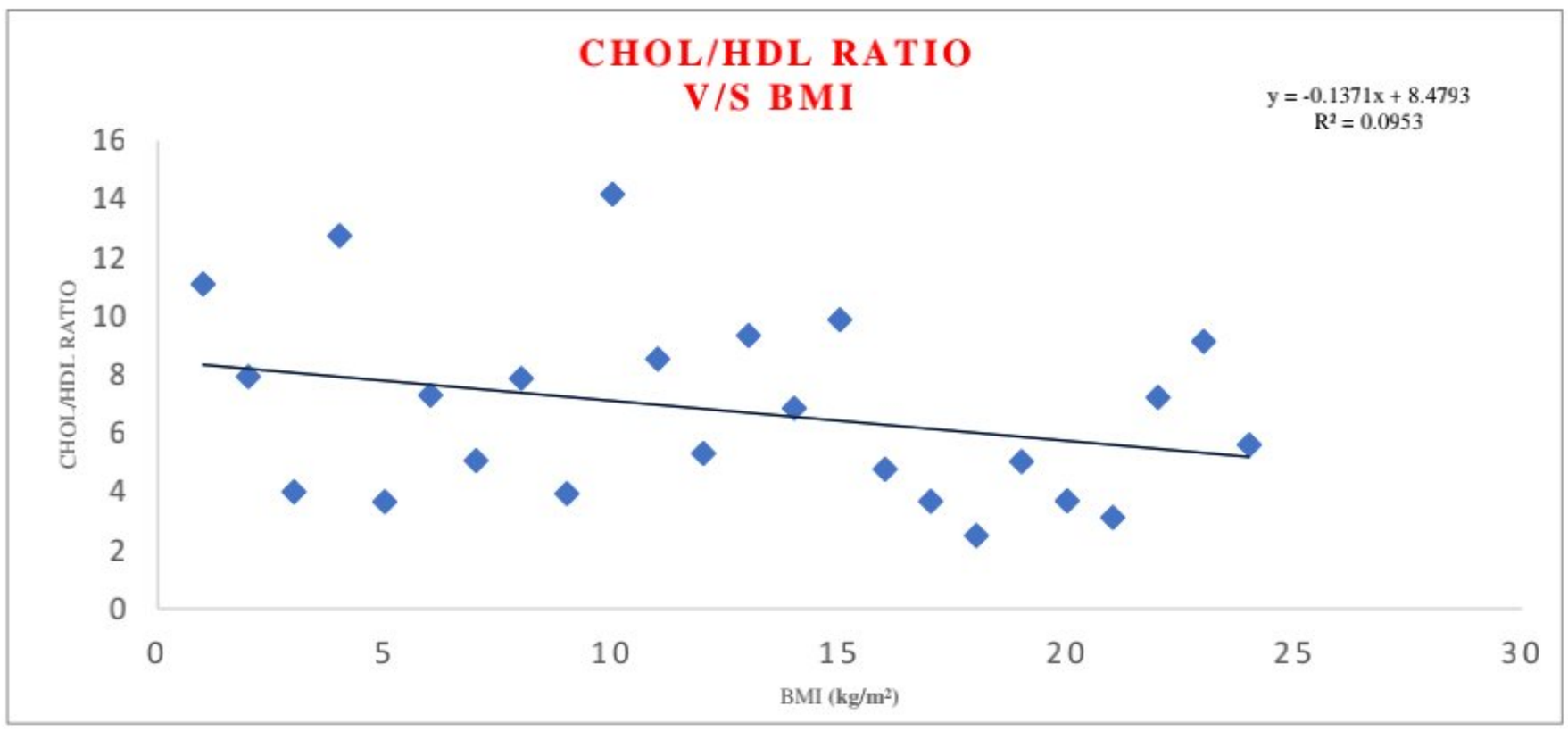


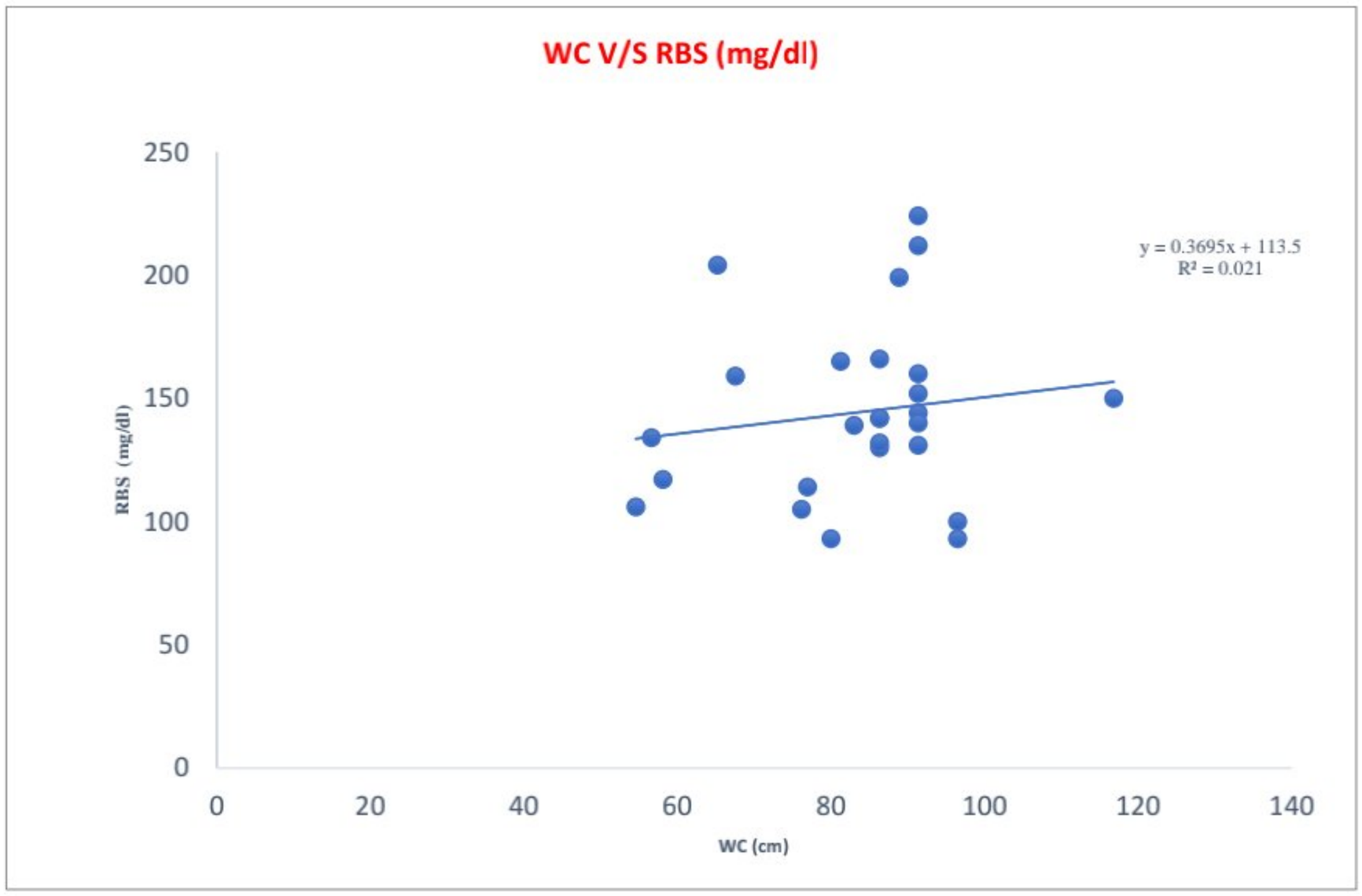
Table No. 9 - Bivariate correlation between WC and Biochemical parameters in SCZ group

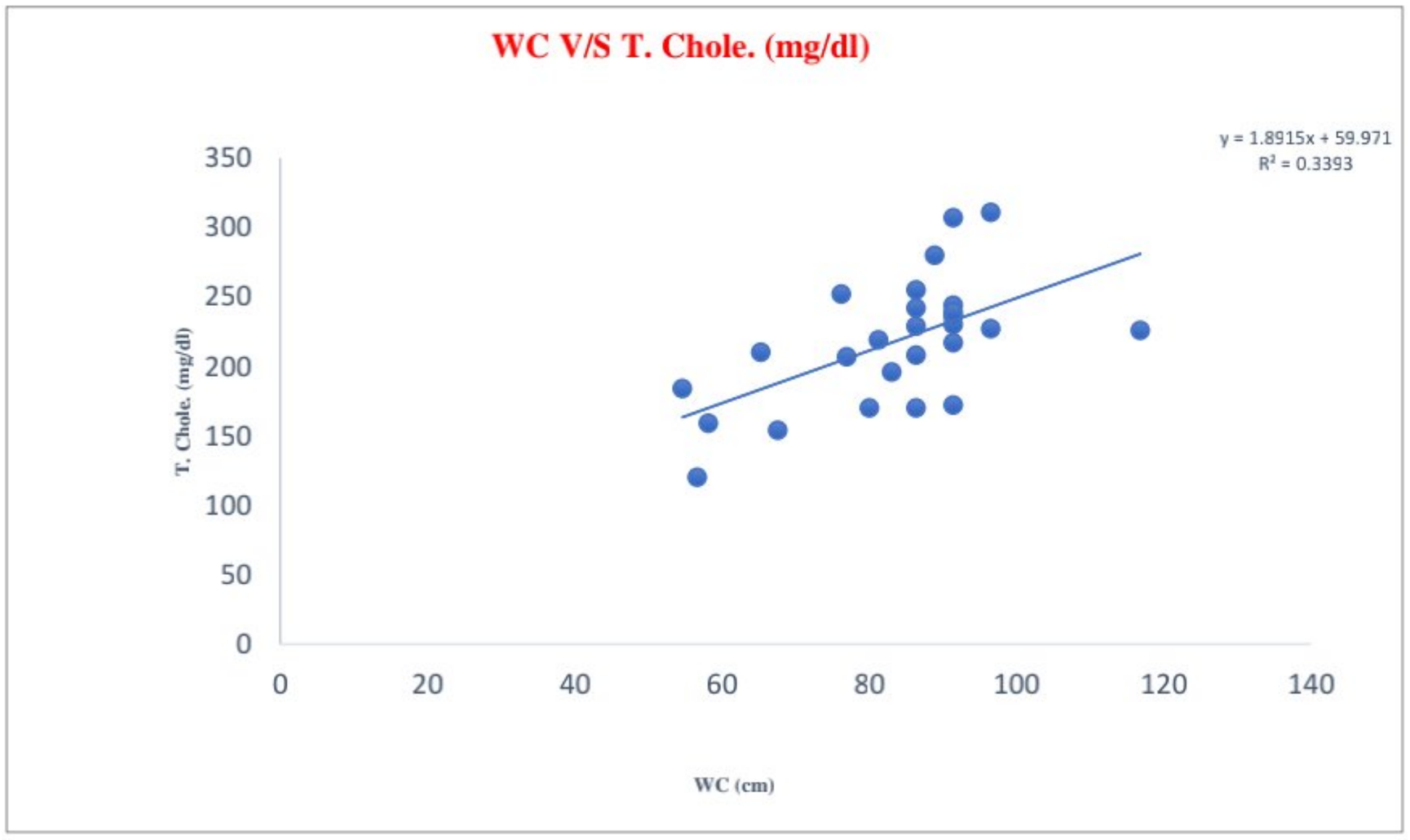
Biochemical parameters	WC	
	r- Value	p-Value
RBS (mg/dl)	0.160	0.455
Serum TC (mg/dl)	0.502*	0.012
Serum TG (mg/dl)	0.342	0.102
Direct HDL-C (mg/dl)	0.240	0.259
Serum LDL-C (mg/dl)	0.244	0.251
Serum VLDL-C (mg/dl)	0.342	0.102
WC V/S TG/HDL	0.245	0.249
WC V/S LDL/HDL	0.085	0.692
WC V/S CHOL/HDL	0.264	0.212

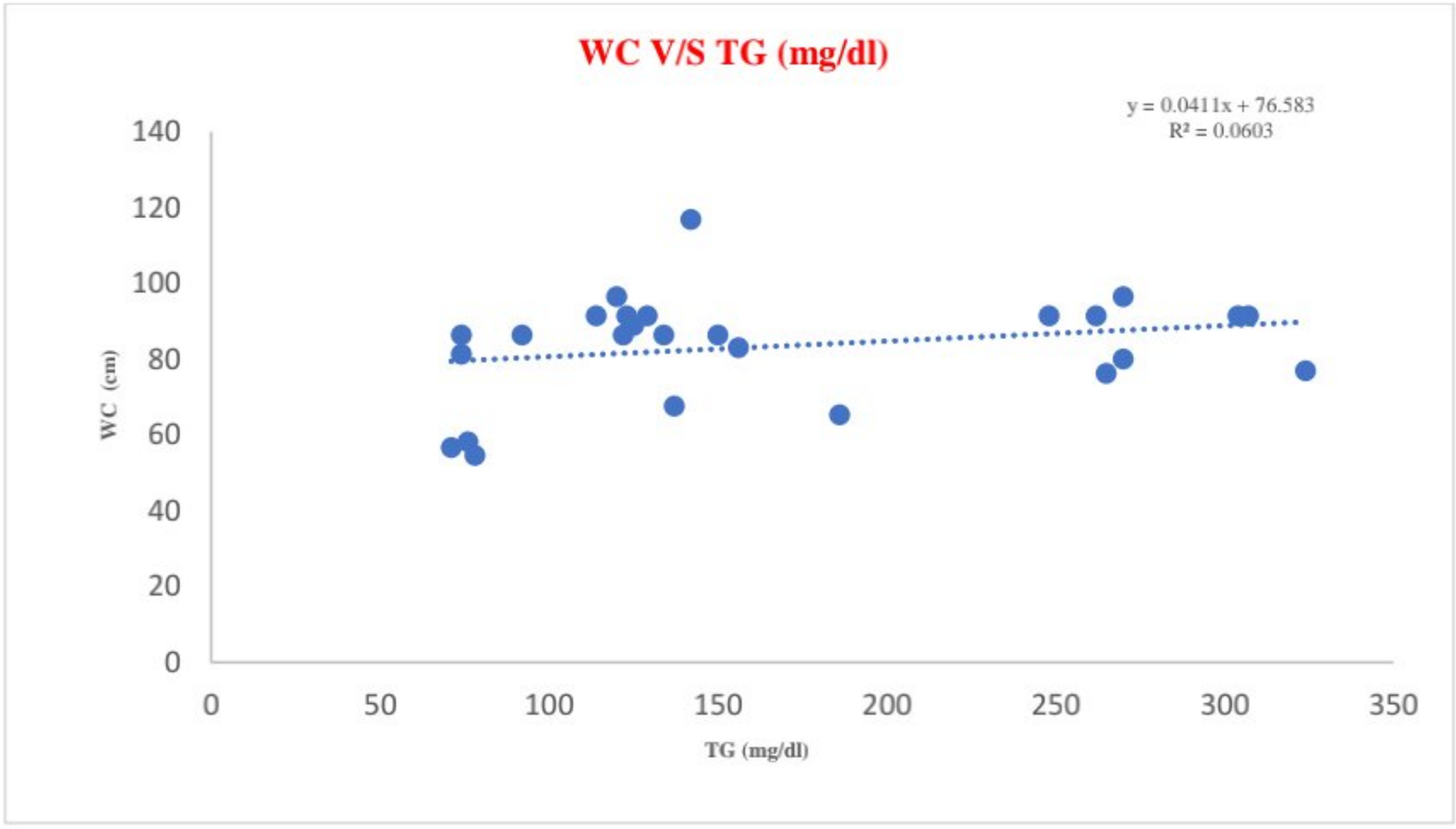
Table No. 9 shows a WC and biochemical parameters such as blood glucose and lipid profile levels had a significant positive correlation, except Direct HDL-C significantly

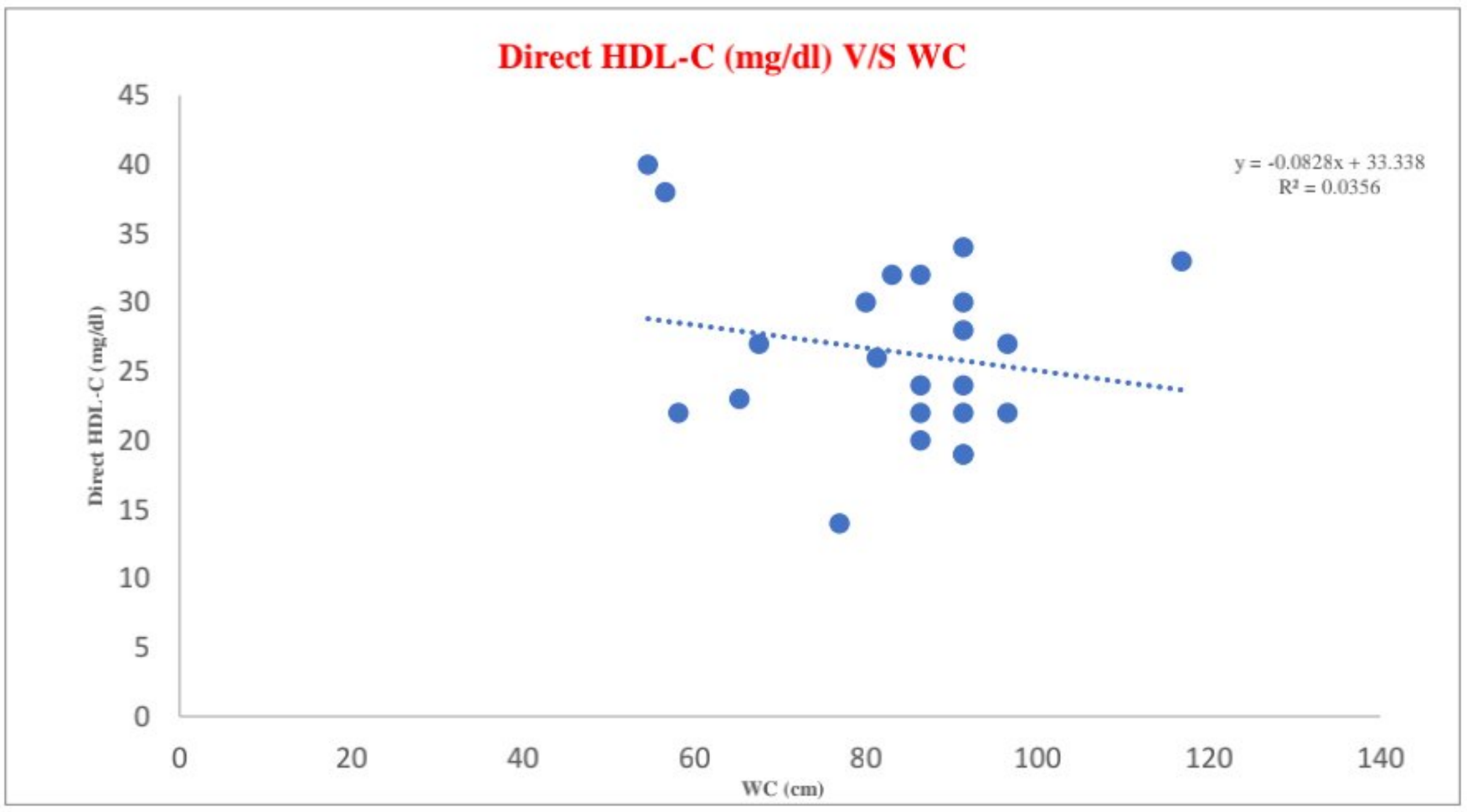
negative correlation, whereas lipid ratios had a positive correlation with WC (WC v/s TG/HDL r : 0.245, p : 0.249. WC v/s LDL/HDL r : 0.085, p : 0.692. WC v/s CHOL/HDL r : 0.264, p : 0.212).

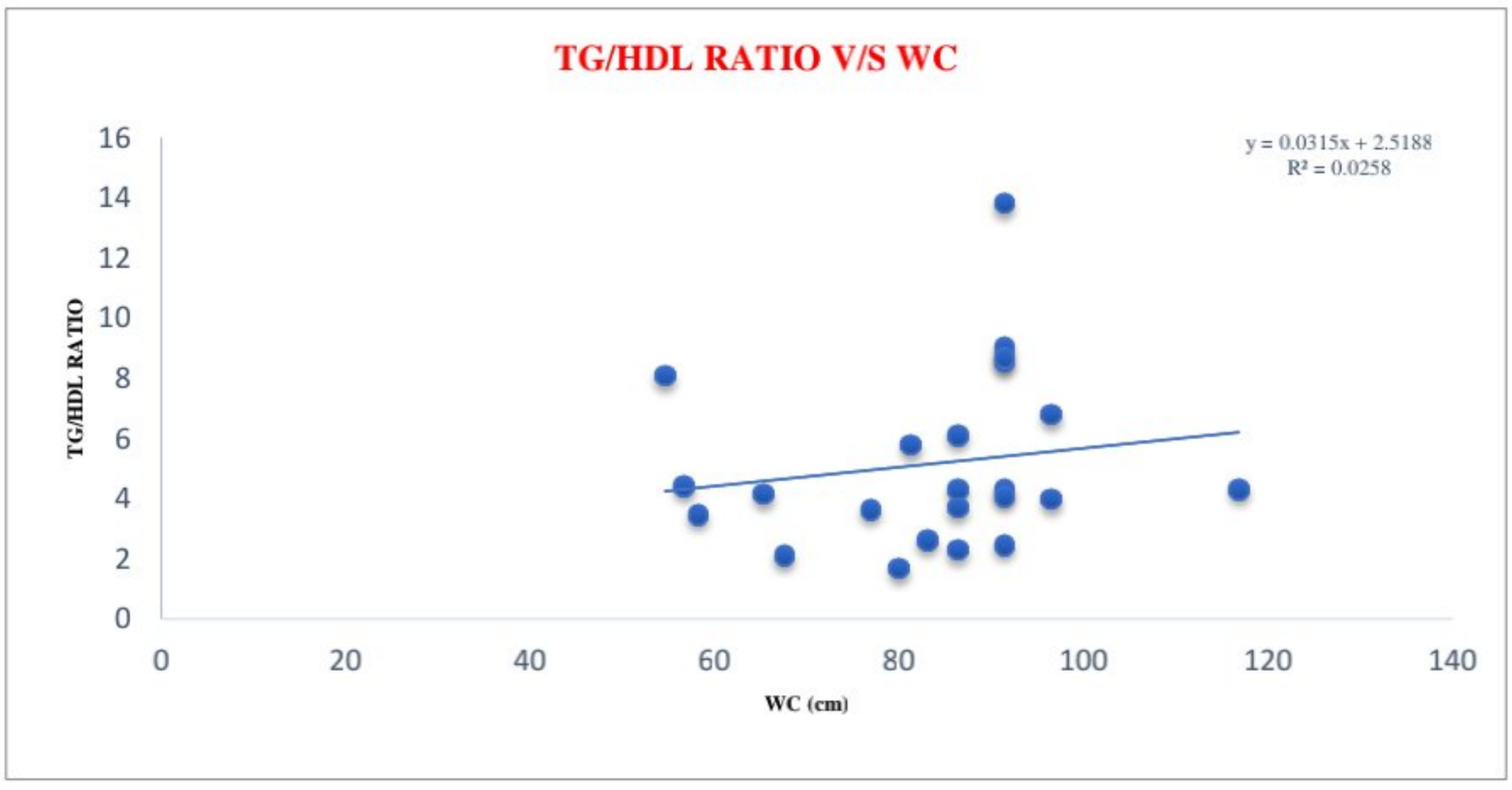
Fig No. 8 Graph Showing Bivariate correlation between WC and Biochemical parameters in SCZ group

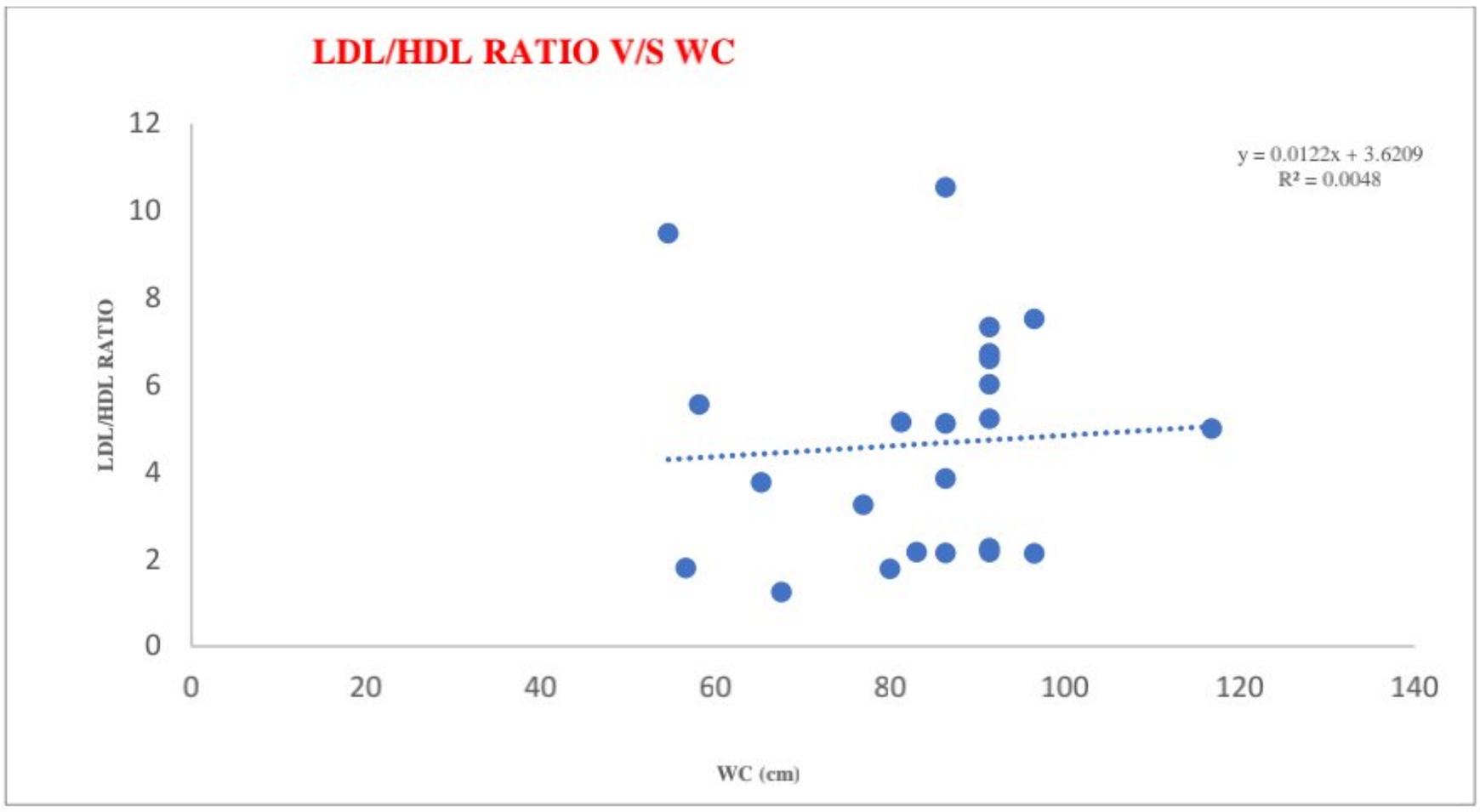


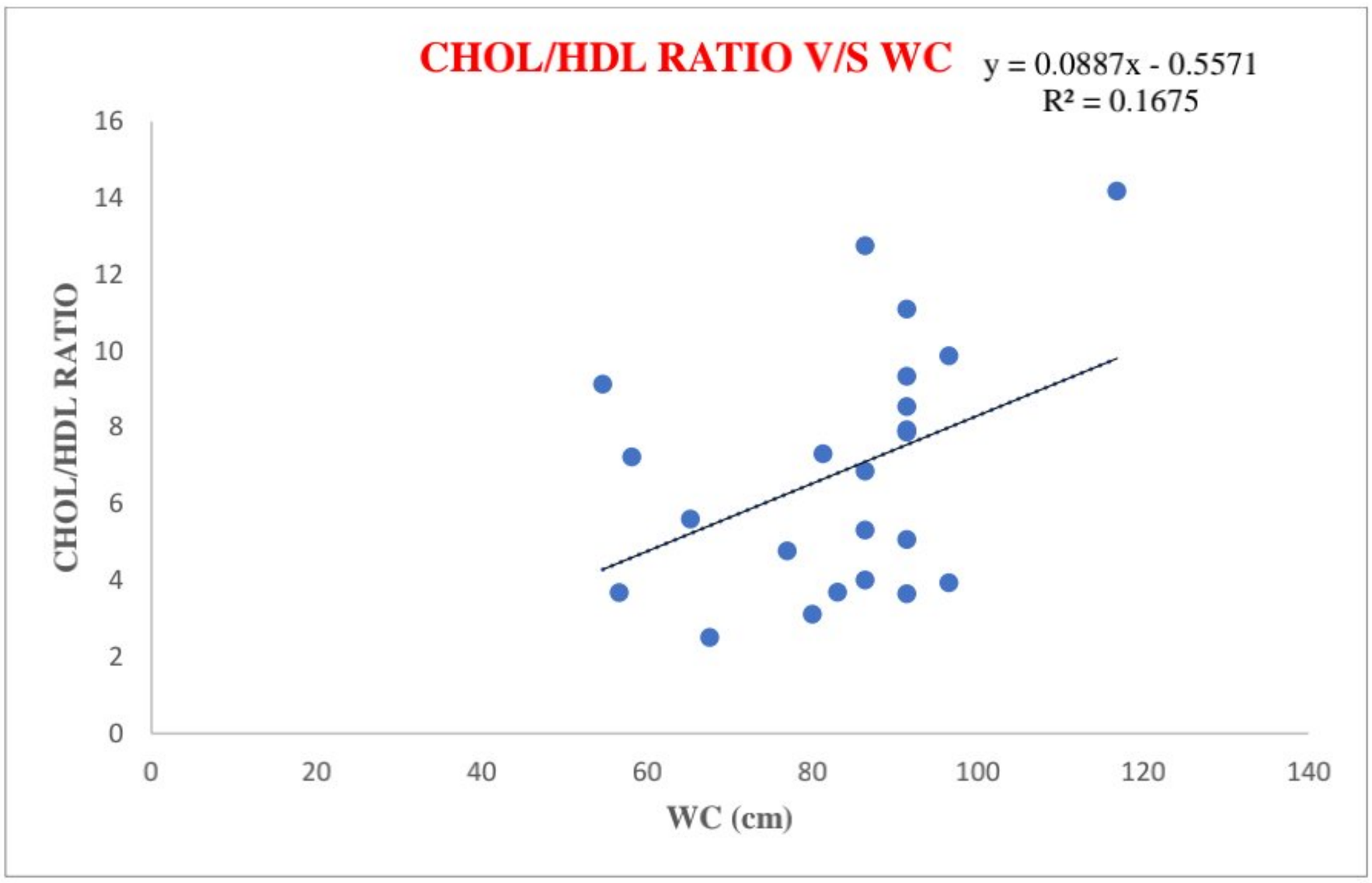



WC V/S TG (mg/dl)
y = 0.0411x + 76.583
R² = 0.0603
WC (cm)
TG (mg/dl)
0
20
40
60
80
100
120
140
0
50
100
150
200
250
300
350


Direct HDL-C (mg/dl) V/S WC
y = -0.0828x + 33.338
R² = 0.0356
Direct HDL-C (mg/dl)
WC (cm)
0
5
10
15
20
25
30
35
40
45
0
20
40
60
80
100
120
140

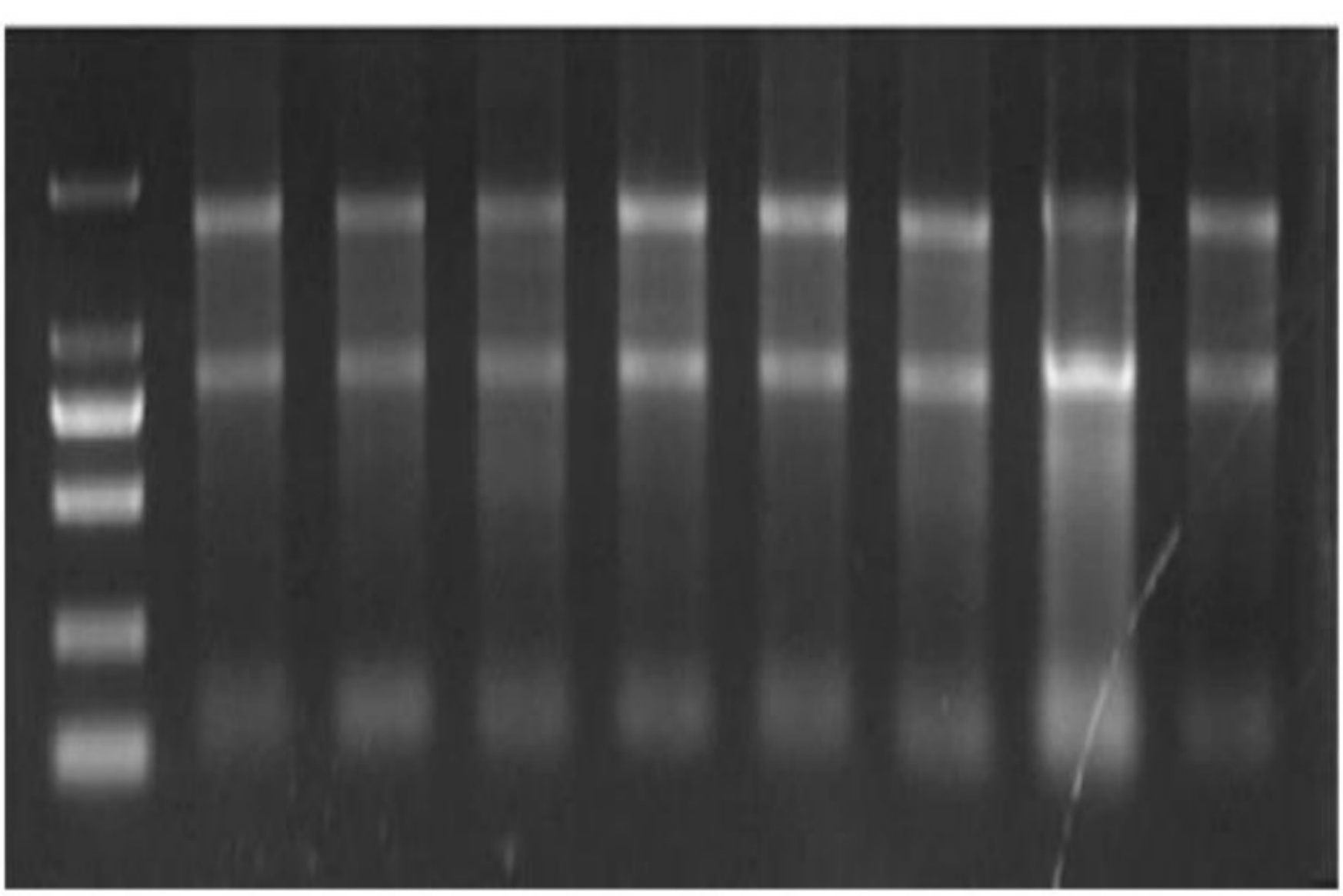

TG/HDL RATIO V/S WC
y = 0.0315x + 2.5188
R² = 0.0258
TG/HDL RATIO
WC (cm)
0
2
4
6
8
10
12
14
16
0
20
40
60
80
100
120
140


LDL/HDL RATIO V/S WC
y = 0.0122x + 3.6209
R² = 0.0048
LDL/HDL RATIO
12
10
8
6
4
2
0
0
20
40
60
80
100
120
140
WC (cm)


CHOL/HDL RATIO V/S WC
y = 0.0887x - 0.5571
R² = 0.1675
CHOL/HDL RATIO
16
14
12
10
8
6
4
2
0
0
20
40
60
80
100
120
140
WC (cm)

RELN gene expression in SCZ :

Using the standard protocol as mentioned in materials and methods Isolation of RNA from whole blood was done by TRIZOL method :



RNA extract Bands are clearly seen in lane 1-8

Quantification :

Table No. 10 RT- PCR Analysis results value of study group and control group :

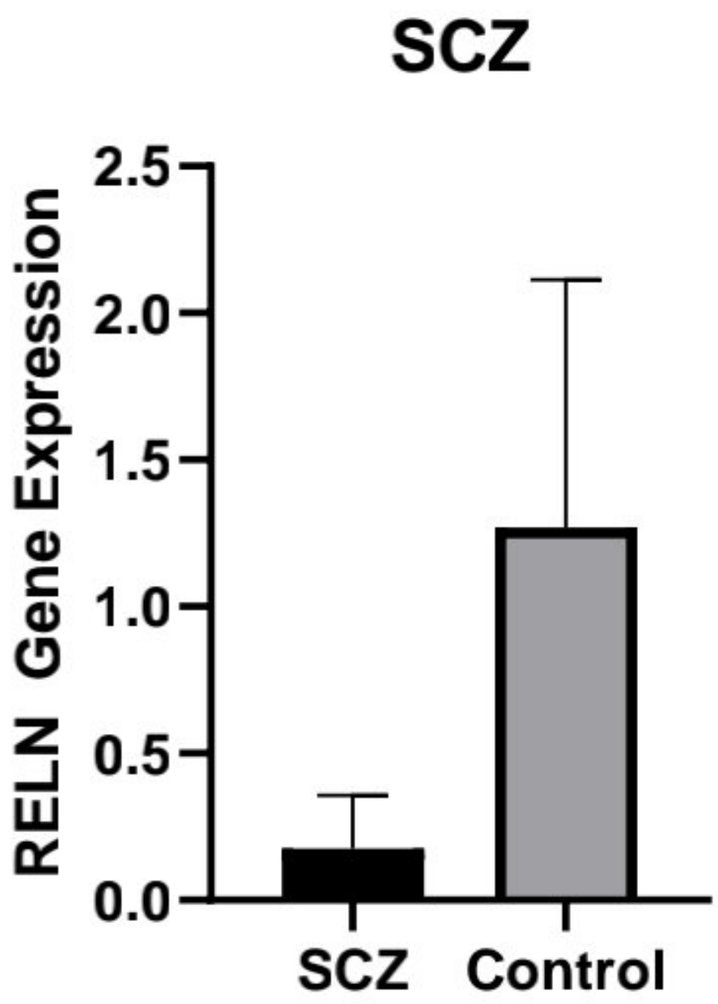
Study Groups :

Case No.	2^ - ΔΔCT	Case No.	2^ - ΔΔCT
1	0.044515	35	1.811896
2	0.004335	36	0.217949
3	0.008318	37	0.027978
4	0.035169	38	5.939981
5	0.174398	39	495.2684
6	0.013701	40	13.83699
7	0.065174	41	23.54747
8	0.170809	42	0.274999
9	0.228530	43	0.400662
s	6.278667	44	13.85243
11	0.093915	45	13.43105
12	0.529267	46	14.22600
13	0.016539	47	1.591578
14	0.005952	48	14.74411
15	2.829325	49	152.4367
16	12.38445	50	2.755027
17	0.007824	51	4.279671
18	0.003649	52	0.543540
19	0.002358	53	0.357868
20	0.264254	54	0.417676
21	0.001373		
22	0.005226		
23	0.115860		
24	0.947414		
25	0.748496		
26	0.136830		
27	82.13925		
28	0.478802		
29	0.926882		
30	13.15464		
31	4.748593		
32	3.816015		
33	245.1464		

Control Group :

Control No.	2^ - ΔΔCT	Control No.	2^ - ΔΔCT	Control No.	2^ - ΔΔCT
55	50.57870	79	0.799682	103	35.80444
56	0.001575	80	0.416821	104	86.34655
57	0.438442	81	0.006247	105	0.00639
58	0.015848	82	89.10904	106	0.017579
59	0.001004	83	0.061107	107	232.3249
60	0.784833	84	1.471716	108	0.244855
61	61.83956	85	0.449845		
62	26.91723	86	0.032974		
63	0.517796	87	83.71997		
64	7.461461	88	23.38481		
65	69.09255	89	0.044117		
66	1.102255	90	46.76962		
67	41.65617	91	43.47356		
68	27.48282	92	0.001354		
69	0.007444	93	0.376851		
70	1.283833	94	0.013621		
71	0.008090	95	0.000863		
72	19.97963	96	0.674582		
73	0.112692	97	9.396129		
74	0.554961	98	190.2914		
75	0.379049	99	0.445058		
76	28.45200	100	6.413298		
77	0.181804	101	59.38664		
78	21.56258	102	0.947413		

Figure No. 9 : Graph showing an expression of RELN gene



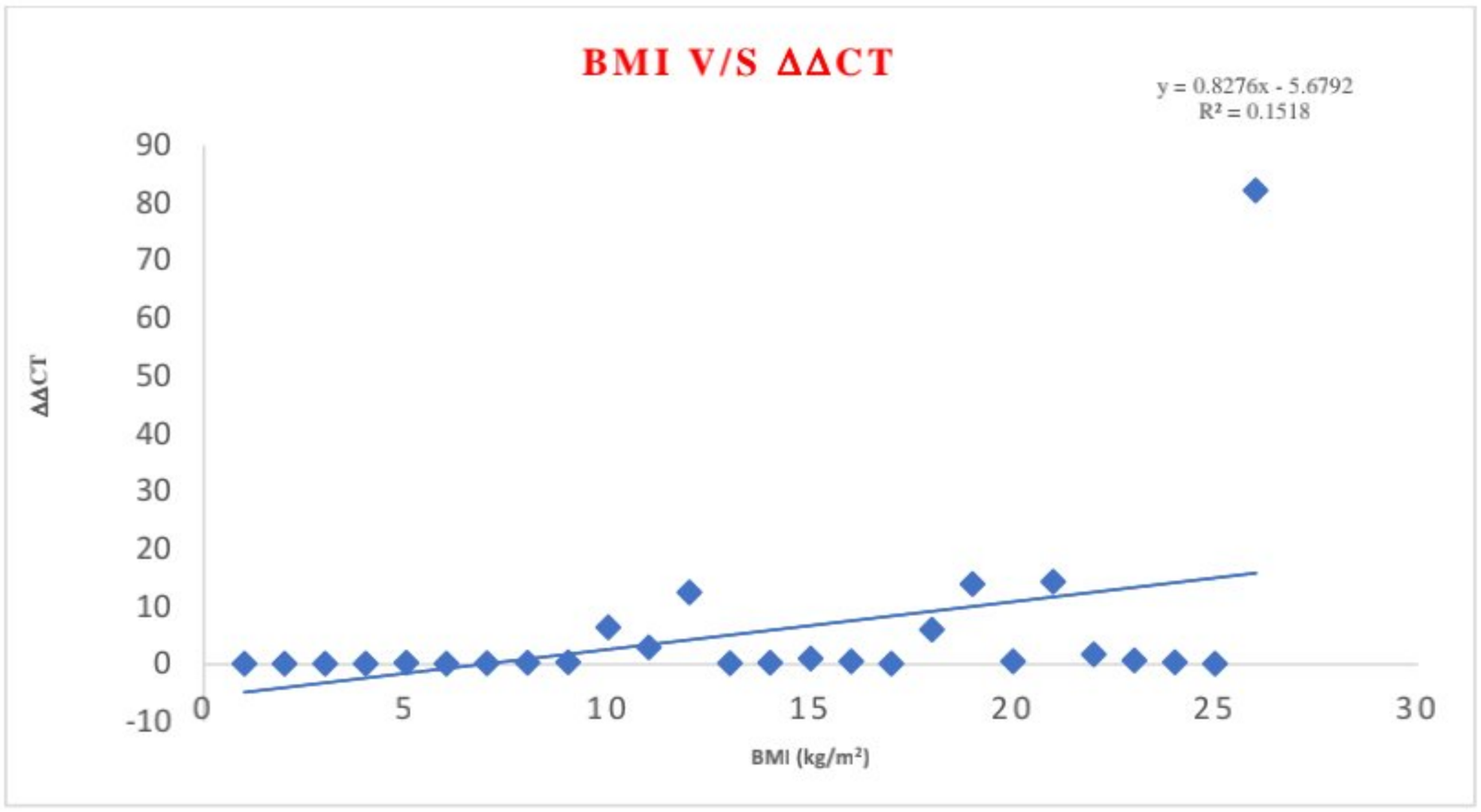
RT-PCR results revealed a decrease in RELN gene expression in schizophrenia patients compared to a control group ranging in age group from 20 to 65 years.

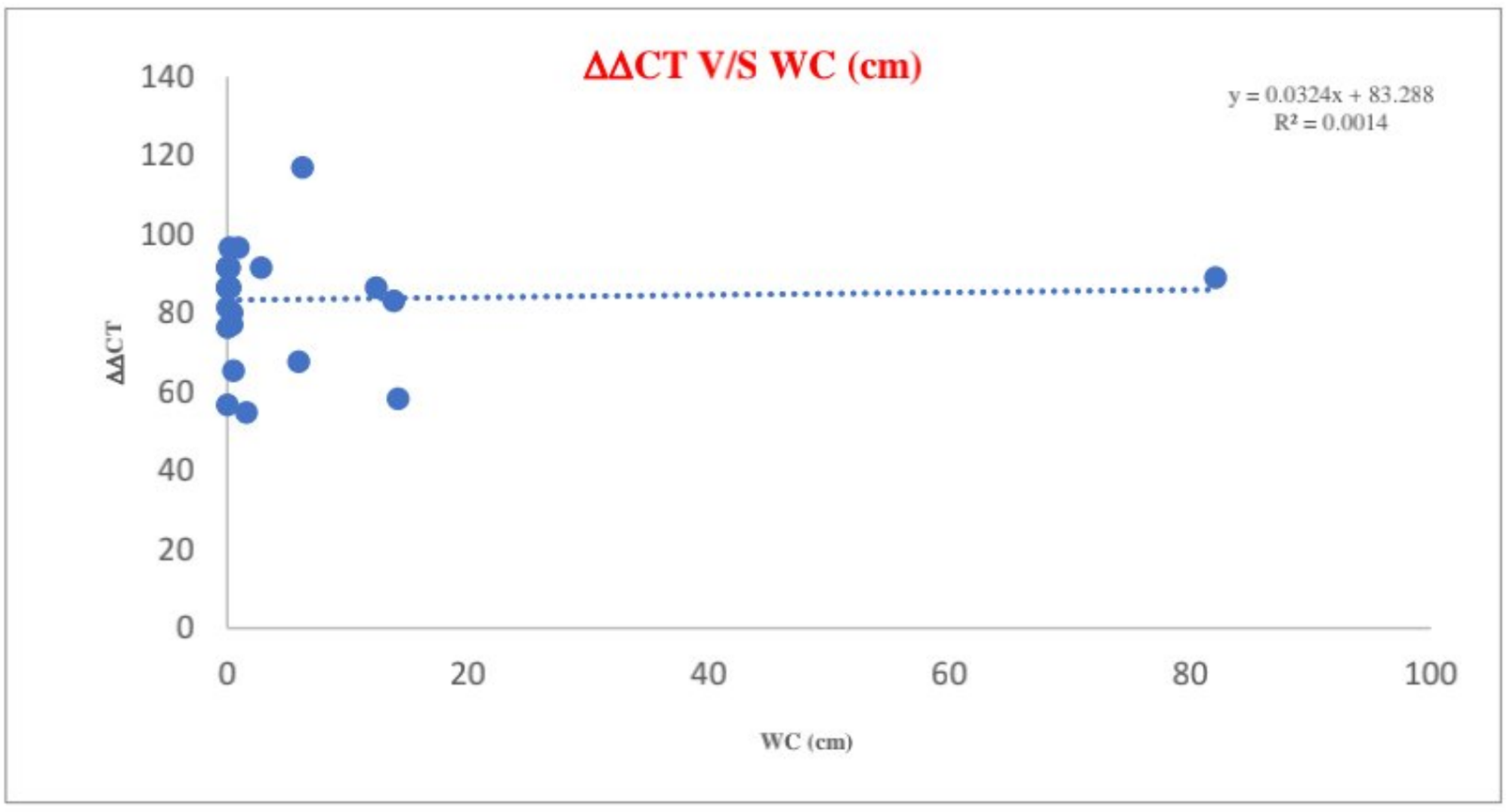
Table No. 11 - Bivariate correlation between RELN gene expression level with BMI, WC, and Biochemical parameters in SCZ group

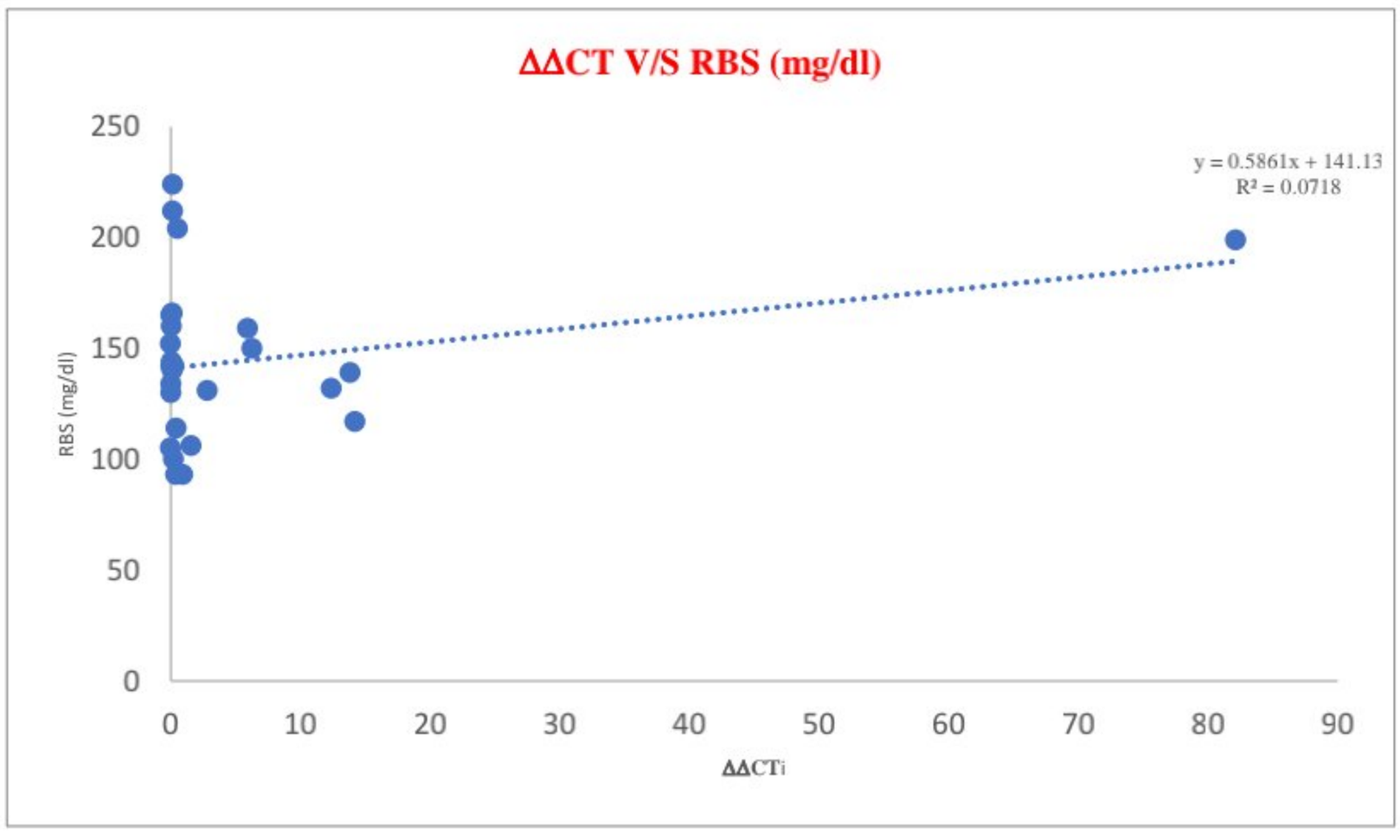
	RELN gene Expression	
	r- Value	**p-Value**
BMI	0.345	0.085
WC	-0.141	0.491
RBS (mg/dl)	-0.084	0.676
Serum TC (mg/dl)	-0.319	0.128
Serum TG (mg/dl)	-0.373	0.073
Direct HDL-C (mg/dl)	0.377	0.070
Serum LDL-C (mg/dl)	-0.717	0.000
Serum VLDL-C (mg/dl)	-0.364	0.067
ΔΔCT V/S TG/HDL	-0.690	0.000
ΔΔCT V/S LDL/HDL	-0.917	0.000
ΔΔCT V/S CHOL/HDL	-1.000**	0.000

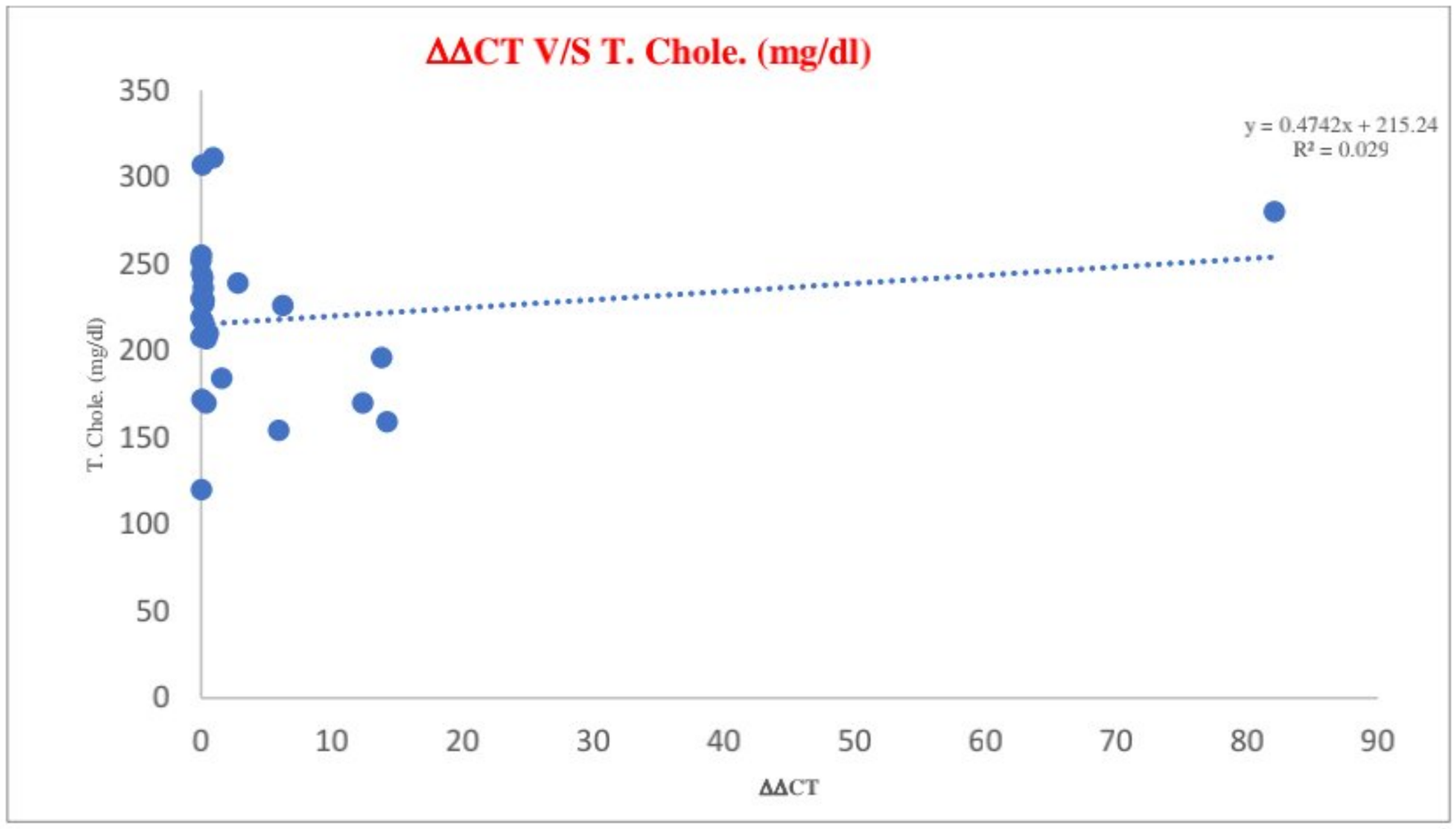
ΔΔCT expression level was significantly positively correlated with BMI, while WC, blood glucose, lipid profile except for HDL-C level, and lipid ratios were significantly negatively correlated with ΔΔCT expression level.

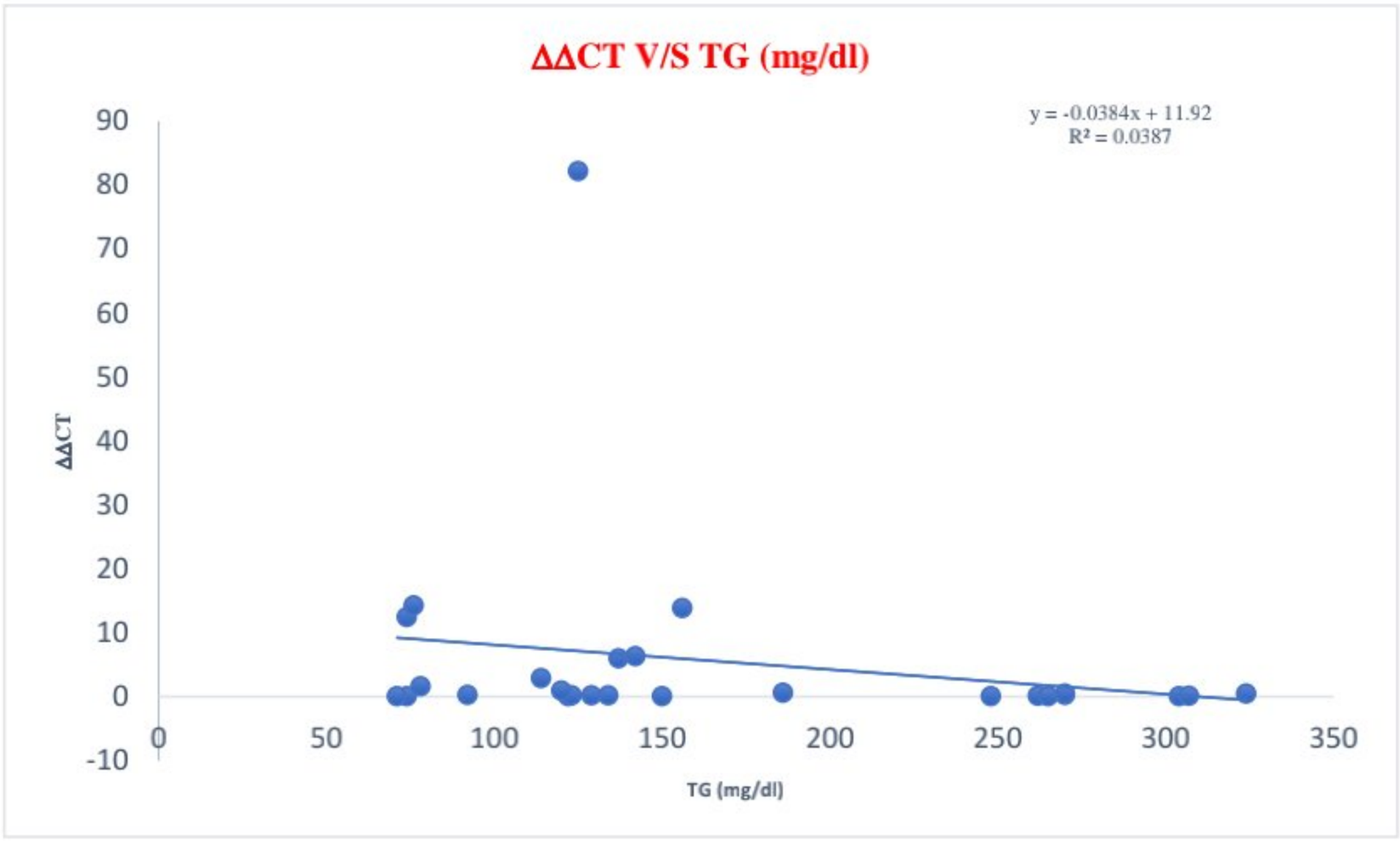
Fig No. 10 Graph Showing Bivariate correlation between RELN gene expression level with BMI, WC, and Biochemical parameters in SCZ group

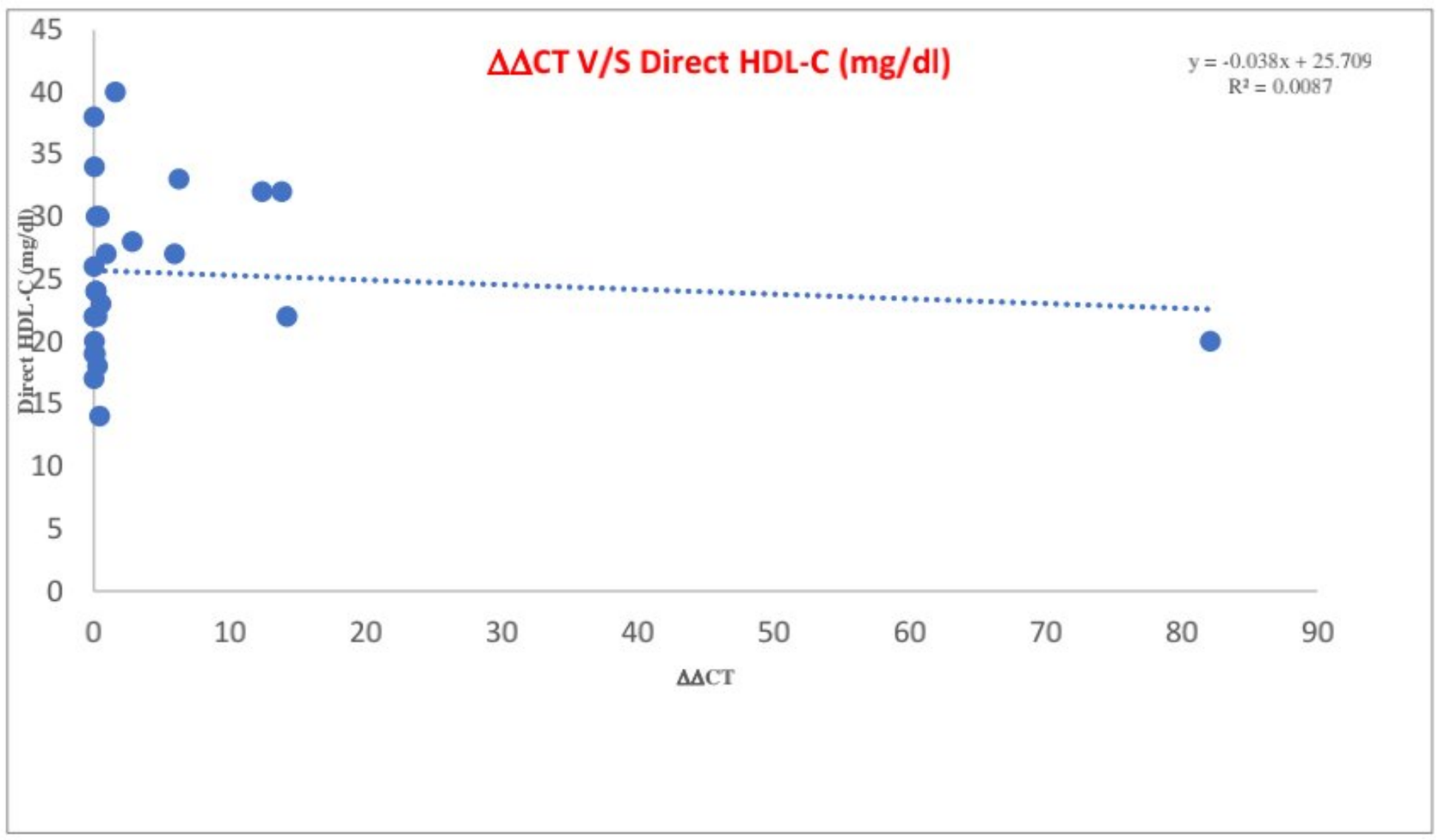


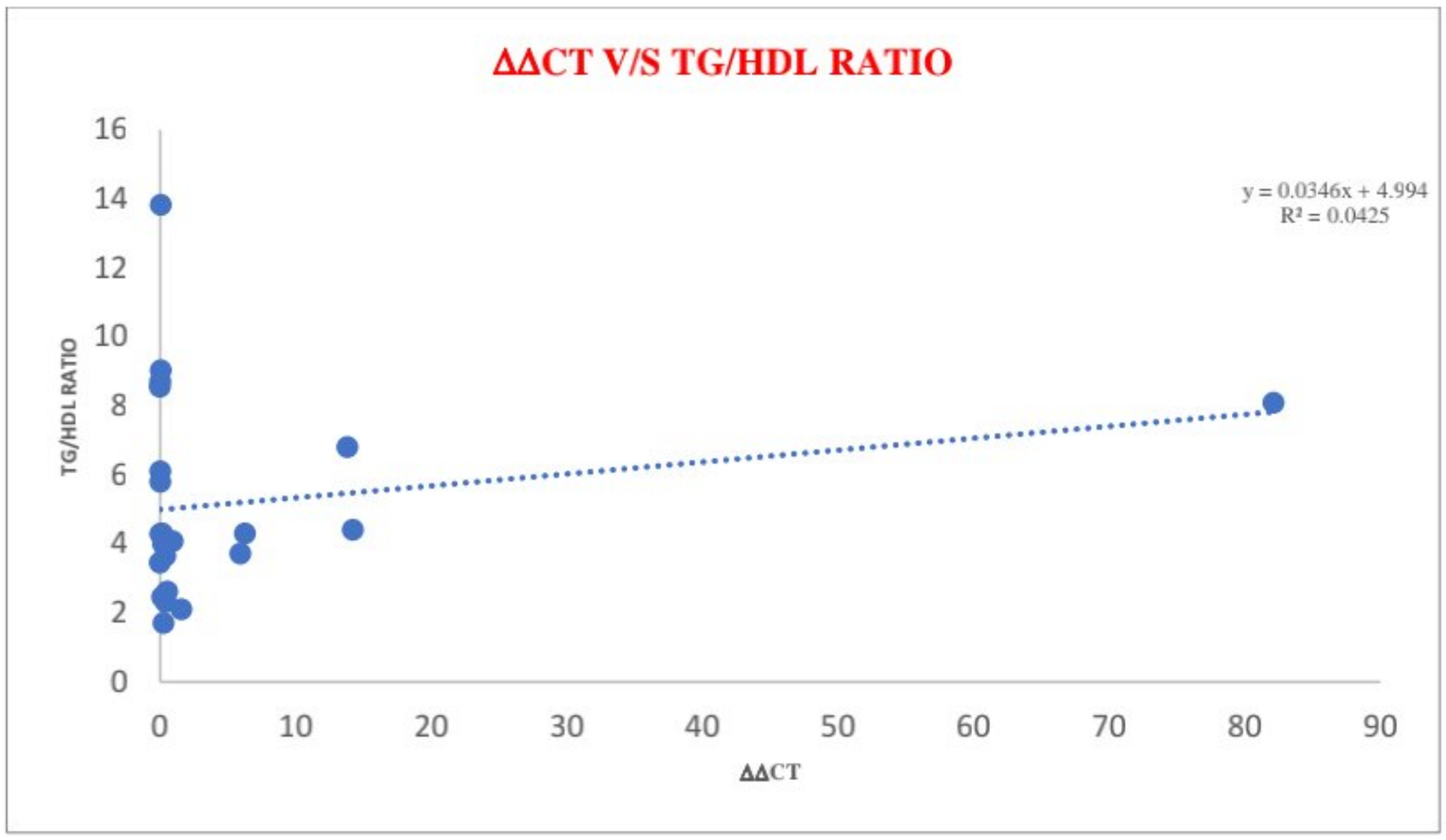


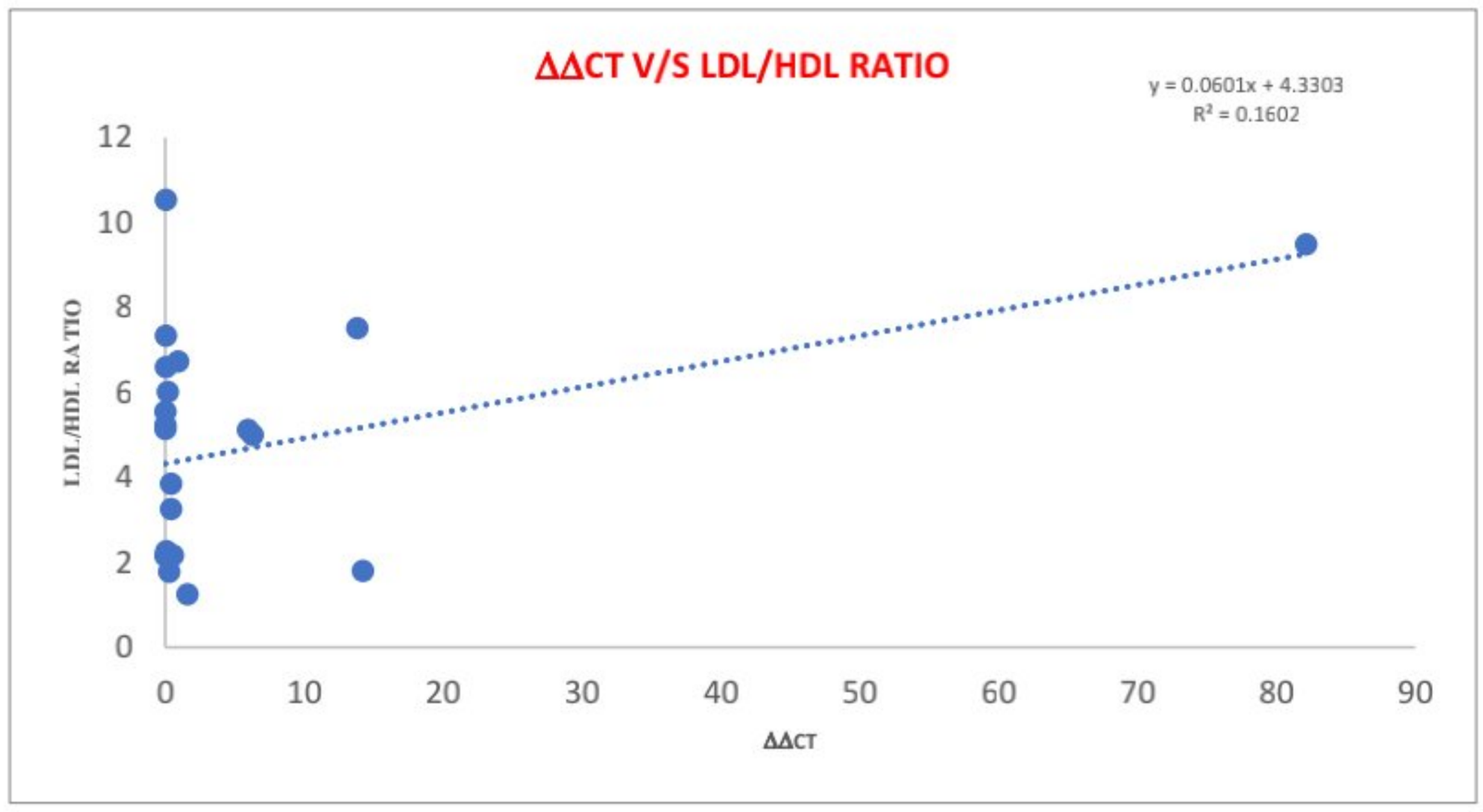


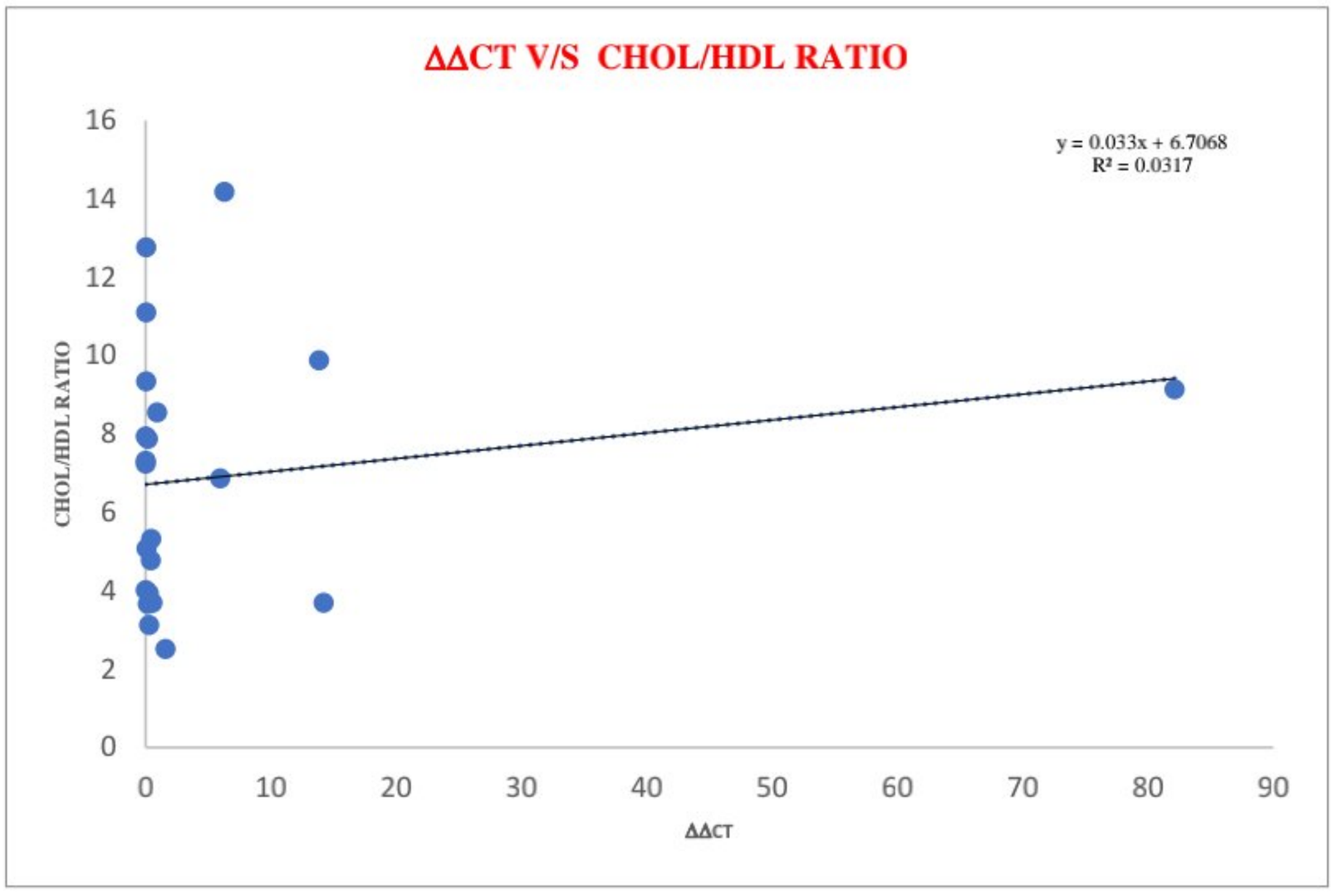

ΔΔCT V/S T. Chole. (mg/dl)
y = 0.4742x + 215.24
R² = 0.029
T. Chole. (mg/dl)
350
300
250
200
150
100
50
0
0
10
20
30
40
50
60
70
80
90
ΔΔCT


ΔΔCT V/S TG (mg/dl)
y = -0.0384x + 11.92
R² = 0.0387
ΔΔCT
90
80
70
60
50
40
30
20
10
0
-10
0
50
100
150
200
250
300
350
TG (mg/dl)


ΔΔCT V/S Direct HDL-C (mg/dl)
y = -0.038x + 25.709
R² = 0.0087
Direct HDL-C (mg/dl)
45
40
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
70
80
90
ΔΔCT


ΔΔCT V/S TG/HDL RATIO
y = 0.0346x + 4.994
R² = 0.0425
TG/HDL RATIO
ΔΔCT
0
2
4
6
8
10
12
14
16
0
10
20
30
40
50
60
70
80
90


ΔΔCT V/S LDL/HDL RATIO
y = 0.0601x + 4.3303
R² = 0.1602
LDL/HDL RATIO
ΔΔCT
0
2
4
6
8
10
12
0
10
20
30
40
50
60
70
80
90


ΔΔCT V/S CHOL/HDL RATIO
y = 0.033x + 6.7068
R² = 0.0317
CHOL/HDL RATIO
ΔΔCT
0
2
4
6
8
10
12
14
16
0
10
20
30
40
50
60
70
80
90

Discussion

Chapter – VI

VI. Discussion :

SCZ is chronic mental disorders characterized by decline in patient's ability to think, feel and behave.. It is a complex condition that can cause various symptoms, including hallucinations, delusions, disorganized speech and behaviour, and negative symptoms, such as social withdrawal and lack of motivation. In the case of schizophrenia, the global prevalence rate is estimated to be approximately 1%. However, the prevalence varies widely across different regions and countries, with some studies reporting rates as high as 3% in certain populations. The incidence of schizophrenia is estimated to be around 0.4 to 0.6 per 1000 people per year. [(4)]

This study reports 50% of the participants in the age ranging from 20-65 years suffered from SCZ. On the basis DSM-V and ICD-10 criteria mainly they suffered from positive and negative symptoms. And showed moderate severity on BPRS Scale. The anthropometric parameters were analysed in these participants showed a highly significant differences as compared to control group. The RELN gene expression analysis in these participants showed the decrease in expression as compared to control group.

Expression analysis :

Reelin (RELN) gene is a protein essential for the formation of neurons and the migration of neurons during embryonic development. It also plays a significant role in other aspects of brain development. Hallucinations, delusions, distorted thinking, and strange behaviour are only a few of the symptoms of the mental illness schizophrenia.

Research has suggested that there may be a link between the RELN gene and schizophrenia. Specifically, it has been found that individuals with schizophrenia may have lower levels of RELN in some areas of the brain, such as the prefrontal cortex and hippocampus. Additionally, genetic variations impacting the RELN gene expression have been associated with an increased risk of developing schizophrenia. [(55)]

According to Jiajun Yin et al. (2020), association studies have been conducted in a Chinese population to investigate the genetic correlation of RELN with SCZ. This study showed that RELN mRNA expression was decreased in the whole blood of untreated SCZ patients. These findings are consistent with other research showing that RELN gene expression is down-regulated in the brain and peripheral blood of SCZ patients. The study also showed that 12 weeks of antipsychotic therapy dramatically increased the expression

level of RELN mRNA in SCZ patients. Accordingly, no association studies related to RELN are available from the Indian population. Therefore, the primary objective of the present study is to investigate the association of RELN and SCZ in an Indian population. In another study, M. V. Alfimova et al. (2018). Investigated this relationship. In schizophrenia patients showed greater peripheral blood methylation levels in the RELN gene promoter than healthy controls. Also, it was demonstrated that RELN methylation levels were linked to worse cognitive performance, specifically in working memory and attention.(103)

Similarly, in the our study, we found that the genetic variations impacting the RELN gene mRNA expression decreased in study group compared to control group because of antipsychotic medications. The study revealed that the RELN mRNA expression levels are not statistically significant in SCZ patients after antipsychotic treatment..

Elisa Brietzke et al. (2018), studied, that BMI may be linked to RELN pathway dysregulation. In our study showed a positive correlation between BMI and the expression of the RELN gene compared to the healthy control individual.(104)

According to Gregor Hable et al. (2009), studied, RELN expression is reduced in the left prefrontal cortex (Brodmann area 9 or BA9) in chronic schizophrenia patients. This finding suggests that dysregulation of reelin expression may contribute to the pathophysiology of schizophrenia. Decreased reelin expression in the prefrontal cortex of chronic schizophrenia patients may contribute to this disorder's cognitive and behavioral deficits. In addition, studies have suggested that RELN may play a role in regulating dopamine signaling, which is also involved in the pathophysiology of schizophrenia. Similarly, our study also shows that dysregulation of reelin expression may contribute to the pathophysiology of schizophrenia and suggests that reelin may play a role in regulating dopamine signaling, which is also involved in the pathophysiology of schizophrenia. (105)

In this study, we aimed to determine the expression profiling of the RELN gene in SCZ patients. Apart from gene expression, other factors of metabolic syndrome Diabetes, Cardiovascular diseases, dyslipidemia, obesity, and physical inactivity significantly increase with the antipsychotic treatment in Schizophrenia subjects. These factors are strongly associated with schizophrenia.

In this study, we observed the mean BMI distribution was in study group 27.45 and control group 24.03. The mean BMI was increased in study group as compared to control

group. The results were statistically significant (p - <0.001*). Whereas, the mean WC distribution in study group was 59.65 and in control group 58.03.

The mean WC was increased as compared to control group. The results were statistically significant (p - <0.001*). It is reported that antipsychotics drugs increase the Mets in the patients suffering from psychotic disorders including SCZ. This in turn increases the risk for atherosclerotic cardiovascular disease and early death (Miller, Joshua M et al 2010) [106].Our study reported the evidences of metabolic changes like hypercholesterolemia, triglyderidemia, and decreased HDL-C in the precipitants. Also BMI and WC well correlated with the biochemical markers and RELN gene expression. These factors clearly indicate that these are leading to the risk for Mets. Observed in our study.

SCZ Female (54.4%) and male (45.5%) showed risk to have Mets according to standard citeria.. This may be attributed to the use of antipsychotic drugs. It is observed in this study that most of the that participants were on antipsychotics drugs like olanzapine 111.7%, Clozapine 43.2%, Risperidone 18.3% and Quetiapine 18.3% (Fig No.4). There are controversial reports about which drugs are more prone for inducing metabolic changes. Some studies report Olanzapine and clozapine are considered to be greater risk metabolic abnormalities some mention risperidone and quitiapine [114] The exact mechanism underlying the metabolic association of antipsychotics drug is not clear. Many authors hypothesize that it may be multifactorial which could be due to interference with hormonal control of food intake e.g. Leptin gene.

According to Surendra et al.(2013) [107], a substantial correlation exists between body mass index and the prevalence of metabolic syndrome in people with schizophrenia. According to research by Sung-Hwan Kim et al. (2010), abdominal obesity and dyslipidemia were the leading causes of metabolic syndrome, particularly in young mental patients.[108] According to a study by Hussein O. et al. (2015), antipsychotic medication was associated with higher waist circumference and abdominal obesity in the SCZ group.[109] According to Yamin Zhang et al. (2020), SCZ patients using antipsychotics saw substantial changes in their BMI, waist circumference,& lipid profile at baseline, 2, 4, and 6 weeks. [110]

According to the current study, aripiprazole caused a drop in BMI, while olanzapine, risperidone, and quetiapine caused increases. Our results were compared with earlier research, which also showed that the antipsychotics drugs causes weight gain, obesity,

Diabetes, lipid abnormalities was a key contributor to the development of metabolic syndrome in the study group.

This study hypothesized that RELN gene expression would be associated with or without metabolic syndrome (MetS) in patients with schizophrenia. According to P. Uma Devi et al.(2013), research has shown that people with schizophrenia may alter their lipid profile, including elevated levels of triglycerides, low-density lipoprotein (LDL) cholesterol, and total cholesterol, and reduced levels of high-density lipoprotein (HDL) cholesterol. These lipid abnormalities are associated with an increased risk of cardiovascular disease, a leading cause of death among people with schizophrenia. Newer or second-generation antipsychotics generally have a more favourable metabolic profile than older antipsychotics. However, we found that schizophrenia patients with second-generation antipsychotics, such as clozapine and olanzapine, are associated with weight gain and metabolic disturbances, including hypertriglyceridemia and hypercholesterolemia. In this study, all groups reported poorer metabolic profiles, namely a much higher mean glycaemia than was observed in study group compared to the control groups. Although no significant variations in mean glycaemia levels between the positive and negative groups were discovered after intra-group comparison, those with cognitive symptomology showed significantly greater glucose levels. Also, this investigation revealed that 40% of the patients without a history of diabetes had recently identified abnormalities of glucose metabolism (IGT or type 2 diabetes) compared to the general population, schizophrenic patients had a significantly greater prevalence of diabetes. (111)

In the our study, although Random glucose concentrations did differ between the study groups, there was a significant difference in glucose concentrations, with the highest value demonstrated in patients with SCZ subjects ($p<0.001$). Similarly, Aditi Gupta et al.(2013), in estimated lipid and glucose parameters in SCZ subjects, showed significant changes in glucose, triglyceride, LDL cholesterol and HDL cholesterol in patients receiving olanzapine, quetiapine and risperidone. (112)

Hence, similar to other research, we found that increased cholesterol and blood glucose levels enhance the chance of developing metabolic syndrome. Moreover, it has been shown that alterations in insulin sensitivity are a direct cause of the metabolic abnormalities linked to the use of second generation atypical antipsychotics. The increased metabolic risk may result from impaired parasympathetic modulation of β - cell activity

caused by blocking histaminergic and muscarinic receptors. Type 2 diabetes mellitus and lipid abnormalities such as dyslipidemia and hypercholesterolemia are directly caused by SCZ patients' impaired antipsychotic mediation, which leads to metabolic syndrome.

Zhenyu Zhu et.al. (2022) reprted that the lipid parameters abnormalities observed in SCZ may be because of olanzapine reduces the abundance of short chain fatty acids metabolism related microbiome and serotonin and increasing the gene and protein expression of the appetite -related neuropeptide y / agouti related peptide in the hypothalamus which is proven in the animal model [113]

Overall, the expression profile of the RELN gene in schizophrenia has shed light on the gene function in the condition's pathophysiology. However, further research is needed to fully understand the complex mechanisms underlying the relationship between RELN and schizophrenia.

Conclusion

Summary and Conclusion

Schizophrenia (SCZ) is a devastating neuropsychiatric condition of uncertain ethology with significant adverse effects on affected people, their families, and society. Heterogeneous population is seen in India with high degree of inbreeding. This makes it necessary to screen many patients perhaps within each group in order to get a true picture of contribution of RELN mRNA expression level in SCZ. To find out prevalence of Expression level, specific mutation, and many families need to be investigated. Earlier studies report that reelin Gene expression is downregulated and thus makes patient prone for psychotic disorders including SCZ. This gene is upregulated by the antipsychotic drugs. However, we found the decreased expression of the RELN gene who were on treatment of antipsychotic drugs. This may be because of the small number in this study. Hence the exact role of RELN gene in pathophysiology of SCZ cannot be derived. Further studies are necessary to evaluate the role of other genes such as AGO2, DISC1, LDB1, RUNX3, SIGIRR, SLC18A1, NRG1, CHRNB2, PRKAB2, and ZNF74) that could contribute to the development of neurological disorders in Indian families with SCZ susceptibility.

Antipsychotic drugs induce the behavioural changes, eating disorders, physical inactivity which results in the metabolic changes resulting in making them prone for metabolic syndromes, diabetes and cardiovascular diseases at early stages in psychiatric patients leading to decreased life expectancy.

Life style changes and regular monitoring of the patient on antipsychotic drugs is necessary to improve the quality of life, therapeutic interventions to decrease lipid abnormalities will help in improving the life expectancy in the SCZ patients.

Limitations of the Study:

- No of Patients of SCZ in this study is less.
- The RELN gene analysis at the onset of the SCZ and after treatment should have been done.
- The effect Dose and Duration of the Antipsychotic drugs must be considered to establish a possible link between the drugs and metabolic syndrome.

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Annexure - I
Plagiarism Verification Certificate

BLDE (DEEMED TO BE UNIVERSITY)

PLAGIARISM VERIFICATION CERTIFICATE

1. **Name of the Student :** Mr. Chetan S. Shattar **Reg. No. :** 19MSC002
2. **Title of the Dissertation :** "Expression profiling of RELN Gene in schizophrenia."
3. **Department :** Biochemistry
4. **Name of the Guide and Designation** : Dr. Nilima Dongre, Professor, Department of Biochemistry, BLDE (DU), Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura, Karnataka.
5. **Name of the Co-Guide and Designation** : Dr. Santhosh Ramdurg, Associate Professor, Department of Psychiatry, BLDE (DU), Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura, Karnataka.

The above dissertation was verified for Similarity detection. The report is follows :

Software Used : Turnitin **Date :** 12/03/2023

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The report is attached for the review by the student and Guide.

The plagiarism report of the above dissertation has been reviewed by the undersigned. The similarity index is below accepted norms.

The similarity index is above accepted norms, because of the following reasons :

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The dissertation may be considered for submission to the university. The software report is attached.

Nilima. 12/03/23

Professor
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Signature of the Guide
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Dr. Nilima Dongre

Verified by (Signature)
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Signature of the student

Annexure – II
Ethical Clearance Certificate

BLDE
(DEEMED TO BE UNIVERSITY)
Declared as Deemed to be University u/s 3 of UGC Act, 1956
Accredited with 'A' Grade by NAAC (Cycle-2)
The Constituent College
SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

Azadi Ka Amrit Mahotsav

BLDE (DU)/IEC/ 718/2022-23

30/8/2022

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this University met on **Friday, 26th August, 2022 at 3.30 p.m. in the Department of Pharmacology** scrutinizes the Synopsis of Post Graduate Student of BLDE (DU)'s Shri B.M.Patil Medical College Hospital & Research Centre from ethical clearance point of view. After scrutiny, the following original/ corrected and revised version synopsis of the thesis/ research projects has been accorded ethical clearance.

TITLE: "Expression profiling of RELN Gene in Schizophrenia".

NAME OF THE STUDENT/PRINCIPAL INVESTIGATOR: Mr. Chetan S Shattar, MSc Medical Biochemistry.

NAME OF THE GUIDE: Dr. Nilima Dongre, Professor, Dept of Biochemistry.

Dr. Santoshkumar Jeevangi
Chairperson
IEC, BLDE (DU),
VIJAYAPURA

Chairman,
Institutional Ethical Committee,
BLDE (Deemed to be University)
Vijayapura

Dr.Akram A. Naikwadi
Member Secretary
IEC, BLDE (DU),
VIJAYAPURA

MEMBER SECRETARY
Institutional Ethics Committee
BLDE (Deemed to be University)
Vijayapura-586103, Karnataka

Following documents were placed before Ethical Committee for Scrutinization.

- Copy of Synopsis/Research Projects
- Copy of inform consent form
- Any other relevant document

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Annexure – III

Proforma

Patient Information From

Socio-demographic profile : **Sample No. :**

1. Name : ……
2. Age : ……
3. Gender : Male ☐ Female ☐
4. Place of residence : Urban ☐ Rural ☐
5. Religion : Hindu ☐ Christian ☐ Muslim ☐
6. Education : Educated ☐ Uneducated ☐
7. Occupation : Skilled ☐ Semiskilled ☐ Student ☐ Employed ☐ Homemaker ☐
8. Marital Status : Married ☐ Unmarried ☐

Physiological Anthropometry :

1. Weight : …… 2. Height : ……
3. Waist Circumference : …… 4. BMI : ……

Physiological Parameters :

1. Blood Pressure :

Molecular Parameters :

1. Isolation of RNA
2. RT – PCR Analysis

Biochemical Parameters :

Lipid Profile :

1. Total Cholesterol : ……
2. Serum Triglycerides : ……
3. Direct HDL-C : ……
4. Serum LDL-C : ……
5. Serum VLDL-C : ……

Had Schizophrenia and Metabolic Syndrome : Yes ☐ No ☐

Annexure - IV

Assessments of symptoms of psychotic disorder :

NAME: _____ DATE: _____

PATIENT ID#: _____ MD: _____

BRIEF PSYCHIATRIC RATING SCALE (BPRS)

Please enter the score for the term which best describes the patient's condition.

0 = not assessed, 1 = not present, 2 = very mild, 3 = mild, 4 = moderate, 5 = moderately severe, 6 = severe, 7 = extremely severe

1. **SOMATIC CONCERN** Degree of concern over present bodily health. Rate the degree to which physical health is perceived as a problem by the patient, whether complaints have a realistic basis or not. **SCORE** ☐	10. **HOSTILITY** Animosity, contempt, belligerence, disdain for other people outside the interview situation. Rate solely on the basis of the verbal report of feelings and actions of the patient toward others; do not infer hostility from neurotic defenses, anxiety, nor somatic complaints. (*Rate attitude toward interviewer under "uncooperativeness"*). **SCORE** ☐
2. **ANXIETY** Worry, fear, or over-concern for present or future. Rate solely on the basis of verbal report of patient's own subjective experiences. Do not infer anxiety from physical signs or from neurotic defense mechanisms. **SCORE** ☐	11. **SUSPICIOUSNESS** Brief *(delusional or otherwise)* that others have now, or have had in the past, malicious or discriminatory intent toward the patient. On the basis of verbal report, rate only those suspicions which are currently held whether they concern past or present circumstances. **SCORE** ☐
3. **EMOTIONAL WITHDRAWAL** Deficiency in relating to the interviewer and to the interviewer situation. Rate only the degree to which the patient gives the impression of failing to be in emotional contact with other people in the interview situation. **SCORE** ☐	12. **HALLUCINATORY BEHAVIOR** Perceptions without normal external stimulus correspondence. Rate only those experiences which are reported to have occurred within the last week and which are described as distinctly different from the thought and imagery processes of normal people. **SCORE** ☐
4. **CONCEPTUAL DISORGANIZATION** Degree to which the thought processes are confused, disconnected, or disorganized. Rate on the basis of integration of the verbal products of the patient; do not rate on the basis of patient's subjective impression of his own level of functioning. **SCORE** ☐	13. **MOTOR RETARDATION** Reduction in energy level evidenced in slowed movements. Rate on the basis of observed behavior of the patient only; do not rate on the basis of patient's subjective impression of own energy level. **SCORE** ☐
5. **GUILT FEELINGS** Over-concern or remorse for past behavior. Rate on the basis of the patient's subjective experiences of guilt as evidenced by verbal report with appropriate affect; do not infer guilt feelings from depression, anxiety or neurotic defenses. **SCORE** ☐	14. **UNCOOPERATIVENESS** Evidence of resistance, unfriendliness, resentment, and lack of readiness to cooperate with the interviewer. Rate only on the basis of the patient's attitude and responses to the interviewer and the interview situation; do not rate on basis of reported resentment or uncooperativeness outside the interview situation. **SCORE** ☐
6. **TENSION** Physical and motor manifestations of tension "nervousness", and heightened activation level. Tension should be rated solely on the basis of physical signs and motor behavior and not on the basis of subjective experiences of tension reported by the patient. **SCORE** ☐	15. **UNUSUAL THOUGHT CONTENT** Unusual, odd, strange or bizarre thought content. Rate here the degree of unusualness, not the degree of disorganization of thought processes. **SCORE** ☐
7. **MANNERISMS AND POSTURING** Unusual and unnatural motor benavior, the type of motor behavior which causes certain mental patients to stand out in a crowd of normal people. Rate only abnormality of movements; do not rate simple heightened motor activity here. **SCORE** ☐	16. **BLUNTED AFFECT** Reduced emotional tone, apparent lack of normal feeling or involvement. **SCORE** ☐
8. **GRANDIOSITY** Exaggerated self-opinion, conviction of unusual ability or powers. Rate only on the basis of patient's statements about himself or self-in-relation-to-others, not on the basis of his demeanor in the interview situation. **SCORE** ☐	17. **EXCITEMENT** Heightened emotional tone, agitation, increased reactivity. **SCORE** ☐
9. **DEPRESSIVE MOOD** Despondency in mood, sadness. Rate only degree of despondency; do not rate on the basis of inferences concerning depression based upon general retardation and somatic complaints. **SCORE** ☐	18. **DISORIENTATION** Confusion or lack of proper association for person, place or time. **SCORE** ☐

Master Chart - Study Group

SL NO.	PATIENT NAME	AOF	SEX	OCCUPATION	ANTHROPOMETRIC PARAMETERS						SYSTOLIC BLOOD PRESSURE	DIASTOLIC BLOOD PRESSURE	PSYCHOTIC DISORDERS	OTHER PSYCHOTIC DISORDERS			ANTIPSYCHOTIC DRUGS							MOLECULAR ANALYSIS			BIOCHEMICAL ANALYSIS						LIPID RATIOS			SCALE
					WEIGHT (Kg)	HEIGHT (FEET)	HEIGHT (CENTIMETER)	BMI (Kg/M2)	WAIST CIRCUMFERENCE (Inch.)	WAIST CIRCUMFERENCE (CENTIMETERS)			SCHIZOPHRENIA	OTHER PSYCHOTIC DISORDERS	BIPOLAR DISORDERS	PSYCHOSIS DISORDERS	FAMILY HISTORY	CLOZAPINE	OLANZAPINE	RISPERIDONE	QUETIAPINE	ARIPIPRAZOLE	OTHERS	ISOLATION OF RNA	RT - cDNA CONVERSION	RT-PCR ANALYSIS (2^- DDCT)	Glucose (RBS) mg/dl	TOTAL CHOLESTEROL	SERUM TRIGLYCERIDES	DIRECT HDL-C	SERUM LDL-C	SERUM VLDL-C	TG/HDL RATIO	LDL/HDL RATIO	CHOL/HDL RATIO	BRIEF PSYCHIATRIC RATING SCALE
1	Amit B. Girijavgol	40	M	Employed	63.1	5.2	158.4	25.3	36	91.4	150	100	P	A	A	A	N/A	_	_	_	_	10mg/Day	_	✔	✔	0.04452	144	244	304	22	161.2	60.8	13.81818182	7.327272727	11.09090909	Moderate
2	Kasturi S. Aursang	46	F	Housewife	49	4.5	137.1	26.2	36	91.4	130	90	P	A	A	A	N/A	_	50mg/Day	Before 20mg/Day	_	_	_	✔	✔	0.00434	152	230	248	19	161.4	49.6	8.551724138	5.220689655	7.931034483	Moderate
3	Mahananda K.P	36	F	Housewife	45	4.3	131	26.3	34	86.36	160	100	P	A	A	A	N/A	_	10mg/Day	_	_	_	_	✔	✔	0.00832	130	208	150	22	156	30	4.285714286	2.142857143	4	Severe
4	Rahutappa S. M.	30	M	Farmer	61.6	5	152.4	26.6	34	86.36	130	80	P	A	P	A	N/A	_	10mg/Day	_	_	_	40mg/Day	✔	✔	0.03517	142	255	122	20	210.6	24.4	6.1	10.53	12.75	Moderate
5	Mahadevi Nayakodi	46	F	Housewife	43	4.5	137.1	22.9	36	91.4	100	70	P	A	A	A	N/A	_	10mg/Day	_	_	_	_	✔	✔	0.1744	212	217	123	24	168.4	24.6	2.462962963	2.155555556	3.648148148	Moderate
6	Rizawana R. Mulla	32	F	Housewife	35	4.7	143.2	17.15	32	81.28	120	78	P	A	A	A	N/A	0.5mg/Day	15mg/Day	_	_	10mg/Day	_	✔	✔	0.0137	165	219	74	26	178.2	14.8	5.8	5.14	7.3	Severe
7	Sabavva B. Kallur	40	F	Housewife	45	5.1	155.4	18.7	36	91.4	110	76	P	A	A	A	N/A	_	15mg/Day	_	_	_	_	✔	✔	0.06517	160	172	307	34	76.6	61.4	9.029411765	2.252941176	5.058823529	Moderate
8	Ravindra	48	M	Farmer	61	5.0	152.4	26.4	36	91.4	120	90	P	A	A	A	N/A	25mg/Day	_	_	_	_	_	✔	✔	0.17081	224	236	129	30	180.2	25.8	4.3	6.006666667	7.866666667	Severe
9	Shamajbegum Mulla	31	F	Housewife	71	5.1	155.4	29.5	38	96.52	120	80	P	A	A	A	N/A	_	10mg/Day	_	_	_	_	✔	✔	0.22853	100	227	270	22	153	54	4	2.129411765	3.529411765	Moderate
10	Ravi R. Kulkarni	31	M	Business	130	5.3	161.5	50.1	46	116.84	165	105	P	A	P	A	N/A	_	_	_	_	10mg/Day	500mg/Day	✔	✔	6.27867	150	228	142	33	164.8	28.4	4.303030303	4.987878788	14.17393394	Severe
11	Riyaz Gulburga	45	M	Business	82.5	5.0	152.4	35.7	40	101.6	130	90	A	A	P	A	N/A	_	15mg/Day	_	_	_	1mg/Day	✔	✔	0.09392	131	228	132	26	175.6	26.4	5.076923077	6.753846154	8.142857143	Moderate
12	Chinagibhasha Mujawar	28	M	Driver	50.5	4.6	140.2	25.7	36	91.4	130	90	A	P	A	A	N/A	_	_	_	_	_	20mg/Day	✔	✔	0.52927	118	241	188	27	176.4	37.6	6.962962963	6.533333333	8.929629926	Moderate
13	Lalita Pawar	36	F	Housewife	46	4.5	137.1	24.5	34	86.36	110	80	A	P	A	A	N/A	_	_	_	_	_	50mg/Day	✔	✔	0.01654	126	247	229	19	182.4	45.6	5.871794872	4.164102564	6.333333333	Moderate
14	Shivanand A. Gugi	22	M	Student	64	4.8	146.3	30	32	81.28	130	90	A	A	P	A	N/A	_	15mg/Day	_	_	_	_	✔	✔	0.00535	94	205	163	21	151.4	32.6	7.761904762	7.20952381	9.676470588	Mild
15	Mahantesh Patil	43	M	Business	71.5	5.0	152.4	30.9	36	91.4	130	80	P	A	A	A	N/A	_	-	3mg/Day	_	_	_	✔	✔	2.82933	260	239	114	28	188.2	22.8	4.071428571	6.721428571	8.535714286	Moderate
16	Prashant P. Akalwadi	25	M	Employed	43	4.6	140.2	21.9	34	86.36	130	80	P	A	A	A	N/A	_	20mg/Day	-	_	_	_	✔	✔	12.3845	132	170	74	32	123.2	14.8	2.3125	3.85	5.3125	Severe
17	Sarubai A. Talkeri	35	F	Housewife	54	4.8	146.3	25.3	34	86.36	130	80	A	P	A	A	N/A	_	15mg/Day	_	_	_	10mg?Day	✔	✔	0.00782	156	215	128	20	169.4	25.6	4.266666667	5.313333333	7.166666667	Moderate
18	Kausar Nalband	4	F	Housewife	65	4.8	146.3	30.3	36	91.4	110	86	A	P	A	A	N/A	_	15mg/Day	_	_	_	_	✔	✔	0.00365	174	196	126	33	137.8	25.2	2.416666667	1.975	3.458333333	Moderate
19	Rudragouda Biradar	62	M	Business	54	5.4	164.5	20.1	34	86.36	120	80	A	P	A	A	N/A	0.5mg/Day	_	_	_	_	_	✔	✔	0.00236	174	273	180	24	213	36	7.5	8.875	11.375	Moderate
20	Suresh Talkeri	42	M	Farmer	56	4.8	146.3	26.1	34	86.36	90	70	A	A	A	P	N/A	_	_	10mg/Day	_	_	_	✔	✔	0.26425	142	229	92	18	188.6	18.4	4.285714286	4.892857143	7.821428571	Severe
21	Geeta B. P	38	F	Housewife	47	4.8	146.3	21.9	34	86.36	110	80	A	P	A	A	N/A	_	2.5mg/Day	_	_	_	_	✔	✔	0.00137	155	231	113	24	184.4	22.6	4	3.594736842	5.394736842	Severe
22	Sharanappa Hadpad	55	M	Farmer	41	5.0	152.4	17.67	30	76.2	100	70	A	A	A	P	N/A	_	15mg/Day	_	_	_	_	✔	✔	0.00523	105	252	265	17	182	53	3.022727273	2.236363636	0.381489842	Severe
23	Subhash Kondaguli	52	M	Farmer	72	5.2	158.4	28.9	36	91.4	120	80	P	A	A	A	N/A	15mg/Day	_	_	_	_	_	✔	✔	0.11586	140	307	262	19	235.6	52.4	8.703703704	6.592592593	9.333333333	Moderate
24	Jagannath potadar	52	M	Farmer	64	4.9	149.3	28.8	34	86.36	130	90	A	P	A	A	N/A	_	_	_	300mg/Day	_	_	✔	✔	0.94741	123	197	123	33	137.4	24.6	9.034482759	8.124137931	10.5862069	Moderate
25	Gourishankar Hiremath	40	M	Employed	64.2	5.2	158.4	25.7	30	76.2	140	90	A	P	A	A	N/A	_	_	_	_	_	50mg/Day	✔	✔	0.7485	114	250	160	19	198.6	32.4	3.727272727	4.224242424	5.969696697	Moderate
26	Shilpa Hattyal	27	F	Housewife	72	5.6	152.4	31.1	36	91.4	130	90	P	A	A	A	N/A	_	_	_	_	30mg/Day	_	✔	✔	0.13683	166	242	134	24	191.2	26.8	3.714285714	5.114285714	6.857142857	Moderate
27	Savita Seganvi	28	F	Housewife	64	4.8	146.3	30.04	35	88.9	110	80	A	A	A	P	N/A	_	10mg/Day	_	_	_	_	✔	✔	82.1393	199	280	125	20	235	25	4.529411765	3.741176471	5.647058824	Moderate
28	Mahadevi M. Hugar	40	F	Housewife	62	5.0	152.4	26.8	34	86.36	110	70	A	A	P	A	N/A	_	20mg/Day	_	_	_	_	✔	✔	0.4788	158	306	211	21	242.8	42.2	4.342857143	4.285714286	6	Mild
29	Sulochana V. Bilagi	44	F	Housewife	72	4.8	146.3	33.8	34	86.36	130	80	P	A	A	A	N/A	_	15mg/Day	_	_	_	_	✔	✔	0.92688	93	311	120	27	260	24	6.806451613	7.509677419	9.870967742	Mild
30	Gadigappa D. Patil	43	M	Farmer	61.4	5.0	152.4	26.5	34	86.36	120	80	A	A	P	A	Appl.	_	10mg/Day	_	_	_	_	✔	✔	13.1546	153	326	119	17	285.6	23.8	2	1.816666667	3.116666667	Moderate
31	Laxmi S. Patil	35	F	Housewife	60.5	4.7	143.2	29.5	34.5	87.63	130	80	A	A	P	A	Appl.	_	_	_	300mg/Day	_	_	✔	✔	4.74859	116	207	140	28	151	28	5.675675676	5.324324324	6.756756757	Mild
32	Doddavva Magi	50	F	Housewife	77	4.9	149.3	34.6	36	91.4	116	80	A	P	A	A	N/A	_	_	_	_	_	10mg/Day	✔	✔	3.81601	191	317	221	18	254.8	44.2	2.916666667	1.6875	3.738095238	Mild
33	Mallangouda Desai	47	M	Employed	75	5.2	158.4	30.1	34	86.36	110	80	A	A	P	A	N/A	_	_	_	10mg/Day	_	_	✔	✔	245.146	172	290	153	22	227.4	30.6	5.289473684	3.757894737	7.815789474	Severe
34	Wasim sahaji	42	M	Business	86	5.2	158.2	34.5	38	96.52	110	80	P	A	A	A	N/A	200mg/Day	_	_	_	_	_	✔	✔	0.42942	114	207	324	14	128.2	64.8	3.642857143	3.247619048	4.761904762	Severe
35	Radabai Vandakar	65	F	Housewife	61.2	4.8	146.3	28.7	36	91.4	140	90	A	A	P	A	N/A	_	10mg/Day	_	_	_	_	✔	✔	1.8119	133	214	104	32	161.2	20.8	2.818181818	3.140909091	4.704545455	Moderate

No	Name	X	Int	Mahabaleshwar Fish Market	53	F	1:29:5	49	56	Fremont	1:29:5	49	56	Fremont	1:29:5	49	56	Fremont	1:29:5	49	56	Fremont	1:29:5	49	56	Fremont	1:29:5	49	56	Fremont
97	Mahabaleshwar Strawberries	90	Int	Mahabaleshwar Strawberries	1:32:8	44	63	Hillcrest	1:32:8	44	63	Hillcrest	1:32:8	44	63	Hillcrest	1:32:8	44	63	Hillcrest	1:32:8	44	63	Hillcrest	1:32:8	44	63	Hillcrest	1:32:8	44	63	Hillcrest
58	Sujata Deshmukh	33	F	Sujata Deshmukh	2:47	50	74	Stanford	2:47	50	74	Stanford	2:47	50	74	Stanford	2:47	50	74	Stanford	2:47	50	74	Stanford	2:47	50	74	Stanford	2:47	50	74	Stanford
59	Reema Shah	28	F	Reema Shah	2:37	41	64	Hillcrest	2:37	41	64	Hillcrest	2:37	41	64	Hillcrest	2:37	41	64	Hillcrest	2:37	41	64	Hillcrest	2:37	41	64	Hillcrest	2:37	41	64	Hillcrest
60	Laxmikant Mhaske	44	F	Laxmikant Mhaske	2:19	37	53	Stanford	2:19	37	53	Stanford	2:19	37	53	Stanford	2:19	37	53	Stanford	2:19	37	53	Stanford	2:19	37	53	Stanford	2:19	37	53	Stanford
61	Vandana D'Souza	27	F	Vandana D'Souza	2:09	33	51	Fremont	2:09	33	51	Fremont	2:09	33	51	Fremont	2:09	33	51	Fremont	2:09	33	51	Fremont	2:09	33	51	Fremont	2:09	33	51	Fremont
62	Shashank Avvaru	61	M	Shashank Avvaru	1:54	29	46	Hillcrest	1:54	29	46	Hillcrest	1:54	29	46	Hillcrest	1:54	29	46	Hillcrest	1:54	29	46	Hillcrest	1:54	29	46	Hillcrest	1:54	29	46	Hillcrest
63	Shweta Swapnil	39	F	Shweta Swapnil	1:44	27	42	Hillcrest	1:44	27	42	Hillcrest	1:44	27	42	Hillcrest	1:44	27	42	Hillcrest	1:44	27	42	Hillcrest	1:44	27	42	Hillcrest	1:44	27	42	Hillcrest
64	Rathod Vaibhavi	62	F	Rathod Vaibhavi	1:39	25	39	Hillcrest	1:39	25	39	Hillcrest	1:39	25	39	Hillcrest	1:39	25	39	Hillcrest	1:39	25	39	Hillcrest	1:39	25	39	Hillcrest	1:39	25	39	Hillcrest
65	Mahamuni Venkatesh	57	F	Hillcrest	1:34	24	37	Hillcrest	1:34	24	37	Hillcrest	1:34	24	37	Hillcrest	1:34	24	37	Hillcrest	1:34	24	37	Hillcrest	1:34	24	37	Hillcrest	1:34	24	37	Hillcrest
66	Bhuvaneswari Thanjavur	10	F	Hillcrest	1:29	23	35	Hillcrest	1:29	23	35	Hillcrest	1:29	23	35	Hillcrest	1:29	23	35	Hillcrest	1:29	23	35	Hillcrest	1:29	23	35	Hillcrest	1:29	23	35	Hillcrest
67	Lahoda	11	M	Fremont	1:24	22	34	Fremont	1:24	22	34	Fremont	1:24	22	34	Fremont	1:24	22	34	Fremont	1:24	22	34	Fremont	1:24	22	34	Fremont	1:24	22	34	Fremont
68	Shashi Patil	66	F	Hillcrest	1:19	20	31	Hillcrest	1:19	20	31	Hillcrest	1:19	20	31	Hillcrest	1:19	20	31	Hillcrest	1:19	20	31	Hillcrest	1:19	20	31	Hillcrest	1:19	20	31	Hillcrest
69	Ramamoorthy T	45	M	Ramamoorthy T	1:14	19	29	Hillcrest	1:14	19	29	Hillcrest	1:14	19	29	Hillcrest	1:14	19	29	Hillcrest	1:14	19	29	Hillcrest	1:14	19	29	Hillcrest	1:14	19	29	Hillcrest
70	Sudarsanan T	55	M	Fremont	1:09	17	26	Fremont	1:09	17	26	Fremont	1:09	17	26	Fremont	1:09	17	26	Fremont	1:09	17	26	Fremont	1:09	17	26	Fremont	1:09	17	26	Fremont
71	Ramchandani Chelsea	48	F	Hillcrest	1:04	16	24	Hillcrest	1:04	16	24	Hillcrest	1:04	16	24	Hillcrest	1:04	16	24	Hillcrest	1:04	16	24	Hillcrest	1:04	16	24	Hillcrest	1:04	16	24	Hillcrest
72	Shree Iyer	44	F	Stanford	1:00	15	23	Stanford	1:00	15	23	Stanford	1:00	15	23	Stanford	1:00	15	23	Stanford	1:00	15	23	Stanford	1:00	15	23	Stanford	1:00	15	23	Stanford
73	Chaoppa Bhat	53	M	Fremont	0:57	14	21	Fremont	0:57	14	21	Fremont	0:57	14	21	Fremont	0:57	14	21	Fremont	0:57	14	21	Fremont	0:57	14	21	Fremont	0:57	14	21	Fremont
74	Mahadevi P D	44	F	Hillcrest	0:53	13	20	Hillcrest	0:53	13	20	Hillcrest	0:53	13	20	Hillcrest	0:53	13	20	Hillcrest	0:53	13	20	Hillcrest	0:53	13	20	Hillcrest	0:53	13	20	Hillcrest

S. NO.	PATIENT NAME	AGE	SEX	OCCUPATION	ANTHROPOMETRIC PARAMETERS: WEIGHT (Kg)	HEIGHT (FEET)	HEIGHT (CENTIMETER)	BMI (Kg/M2)	WAIST CIRCUMFERENCE (Inch.)	WAIST CIRCUMFERENCE (CENTIMETERS)	SYSTOLIC BLOOD PRESSURE	DIASTOLIC BLOOD PRESSURE	CLINICAL TEST: SCHIZOPHRENIA	DEPRESSIVE DISORDER	BIPOLAR DISORDERS	PSYCHOSIS DISORDERS	FAMILY HISTORY	ANTIPSYCHOTIC DRUGS: CLOZAPINE	OLANZAPINE	RISPERIDONE	QUETIAPINE	ARIPIPRAZOLE	OTHERS	MOLECULAR ANALYSIS: ISOLATION OF RNA	RT - cDNA CONVERSION	RT-PCR ANALYSIS (2^{-} - DDCT)	BIOCHEMICAL ANALYSIS: Glucose (RBS) mg/dl	TOTAL CHOLESTEROL	SERUM TRIGLYCERIDES	DIRECT HDL-C	SERUM LDL-C	SERUM VLDL-C	TC/HDL RATIO	LDL/HDL RATIO	CHOL/HDL RATIO	DR DEP PSYCHIATRIC RATING SCALE	DR DEP PSYCHIATRIC RATING SCALE
1	Ambika Shukla	45	F	Housewife	65	5.3	161.5	24.9	34	86	120	80	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2	Pawan Gupta	42	M	Engineer	78	5.9	179.8	24.1	36	91	130	85	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3	Seema S. Desai	38	F	Teacher	58	5.2	157.5	23.4	31	79	110	70	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
4	Sunita Gandhi	55	F	Retired	70	5.4	164.6	25.8	38	97	140	90	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
5	Rahul Mehta	29	M	Software Engineer	82	6.0	182.9	24.5	37	94	125	82	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
6	Priya Sharma	33	F	Nurse	60	5.4	164.6	22.1	30	76	115	75	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
7	Anil Kumar	50	M	Businessman	85	5.8	176.8	27.2	40	102	145	95	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
8	Neha Singh	27	F	Student	55	5.1	154.9	22.9	29	74	105	68	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
9	Vijay Patel	60	M	Retired	72	5.5	167.6	25.6	39	99	150	92	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
10	Kavita Joshi	40	F	Doctor	62	5.3	161.5	24.0	33	84	118	78	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
11	Rajesh Kumar	47	M	Farmer	75	5.7	173.7	24.9	35	89	128	84	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
12	Sunita Verma	36	F	Accountant	57	5.2	157.5	23.0	30	76	112	72	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
13	Deepak Yadav	31	M	Electrician	80	5.9	179.8	24.7	36	91	132	86	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
14	Meena Kapoor	52	F	Housewife	68	5.4	164.6	25.1	37	94	138	88	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
15	Suresh Reddy	44	M	Lawyer	88	6.0	182.9	26.3	41	104	148	96	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
16	Anjali Desai	39	F	Artist	59	5.3	161.5	22.6	31	79	114	74	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
17	Ramesh Iyer	58	M	Retired	74	5.6	170.7	25.4	38	97	152	94	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
18	Kiran Bedi	34	F	Police Officer	63	5.4	164.6	23.2	32	81	116	76	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
19	Arjun Nair	26	M	Student	68	5.8	176.8	21.8	32	81	108	70	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
20	Lata Mangeshkar	65	F	Singer	55	5.1	154.9	22.9	33	84	160	98	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

103	Mastani	36	M	Employed	80	5.3	161.5	30.8	30.5	77.47	130	80	A	A	A	A	N/A	•	•	[illegible]
104	Rafik	37	M	Employed	73	5.4	164.5	27.2	26	56.34	120	80	A	A	A	A	N/A	•	•	[illegible]
105	Shamia	55	F	Housewife	60	5.2	158.4	24.09	23	58.42	130	70	A	A	A	A	N/A	•	•	[illegible]
106	Sumitra	40	F	Employed	45	5.2	158.4	18.37	17.3	43.94	110	70	A	A	A	A	N/A	•	•	[illegible]
107	Parvati	46	F	Housewife	65	5	152.4	28.1	28	71.12	130	80	A	A	A	A	N/A	•	•	[illegible]
108	[illegible]	50	F	Housewife	62	5.4	164.5	23.1	23.1	56.29	110	90	A	A	A	A	N/A	•	•	[illegible]
109	Mumtaz	43	F	Housewife	60	5	152.4	25.9	25.3	64.26	120	90	A	A	A	A	N/A	x	x	[illegible]
110	Sayara	39	F	Housewife	74	4.8	146.3	34.7	34.6	87.98	110	80	A	A	A	A	N/A	x	x	[illegible]