

Relationship between Insulin Sensitivity and Lipid Status of Hyperglycemic and Normoglycemic Subjects

A Thesis Work

By

Syeda Umme Fahmida Malik

Reg. No.: 2013461001

Session: 2013-14



Submitted to the
Department of Genetic Engineering and Biotechnology
Shahjalal University of Science and Technology, Sylhet
Bangladesh

For partial fulfillment of the requirements
for the degree of

Doctor of Philosophy
in
Genetic Engineering and Biotechnology

Department of Genetic Engineering and Biotechnology
Shahjalal University of Science and Technology, Sylhet, Bangladesh

November, 2022

Copyright© by Syeda Umme Fahmida Malik 2022
All Rights Reserved

Declaration/Certification

The thesis titled “Relationship among Fatness, Insulin Sensitivity and Lipid Status of Hypoglycemic and Normoglycemic Subjects” performed at Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet, submitted by Syeda Umme Fahmida Malik; Registration No. 2013461001, of the Department of Genetic Engineering and Biotechnology, Shahjalal University of Science & Technology, has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of PhD in Genetic Engineering and Biotechnology and approved as to its style and contents.

The work was done under the supervision of **Dr. Md. Abul Kalam Azad** Professor, Department of Genetic Engineering & Biotechnology, Shahjalal University of Science and Technology, Sylhet, Bangladesh, and co-supervision of **Dr. Md. Jahangir Alam** Professor, Department of Genetic Engineering & Biotechnology, Shahjalal University of Science and Technology, Sylhet, Bangladesh.

Syeda Umme Fahmida Malik
Registration No. 2013461001
Department of Genetic Engineering & Biotechnology, Shahjalal University of Science and Technology, Sylhet, Bangladesh.

In the capacity as Supervisor and Co-supervisor of the candidate’s thesis, it is certified that the above statements are true to the best of the knowledge.

Prof. Dr. Md. Abul Kalam Azad
(Supervisor)
Department of Genetic Engineering & Biotechnology,
Shahjalal University of Science and Technology, Sylhet, Bangladesh

Prof. Dr. Md. Jahangir Alam
(Co-supervisor)
Department of Genetic Engineering & Biotechnology,
Shahjalal University of Science and Technology, Sylhet, Bangladesh.

Acknowledgment

I express my gratitude to the Almighty Allah from the core of my heart for blessings, guidance, protection, help, and wisdom in all aspects of my life. All admire goes to Allah who enabled the author to pursue higher education and complete present research work successfully and submit a thesis paper leading to a Doctor of Philosophy degree in Genetic Engineering & Biotechnology.

I am undoubtedly in debt to my beloved parents.

I owe a profound sense of gratefulness to my reverend supervisor **Dr. Md. Abul Kalam Azad**, Professor, Department of Genetic Engineering & Biotechnology, Shahjalal University of Science & Technology, Sylhet, Bangladesh for suggesting the topic of this exposition, his constant encouragement, and precious personal regulation for carrying out my research. I am also grateful to my co-supervisors **Dr. Md. Jahangir Alam**, Professor, Department of Genetic Engineering & Biotechnology, Shahjalal University of Science and Technology, Sylhet, Bangladesh.

I want to express my deep gratitude to **G. M. Nurnabi Azad Jewel**, Assistant Professor, Department of Genetic Engineering & Biotechnology, Shahjalal University of Science and Technology, Sylhet, and **Md. Nazmul Hasan**, PhD Research Fellow, Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet, Bangladesh for their countless support.

I want to express my deep gratitude to all of my respected teachers at the Department of Genetic Engineering and Biotechnology, Shahjalal University of Science & Technology, Sylhet, Bangladesh. I would like to thank all of my friends, and well-wishers, for their contribution in this regard. I am also thankful to the officers and staff of the Department of GEB for their assistance.

I am exclusively responsible for errors and omissions in this exposition if any.

Thank you

The Author

DEDICATION

*I would like to dedicate the thesis to my
beloved parents*

Abstract

Type 2 diabetes mellitus (T2DM) is primarily due to a decreased response to insulin in the tissues of the body, which is defined as the insulin resistance (IR). Excess weight, obesity and morbid obesity are all risk factors for developing T2DM. The usual characteristics of South Asians include low muscle mass, a high body fat percentage, abdominal obesity, insulin resistance, and hyperinsulinemia. Type 2 diabetes is among the most serious consequences of being overweight or obese. The risk factors for cardiovascular illnesses in obese people are insulin resistance (IR) and abnormal lipid profiles. To investigate the relationship among IR, obesity and lipid profile, this study was conducted on a total of 1500 Bangladeshi people at the time of their general health checkup in the North East Medical College Hospital. The Ethical Committee of North East Medical College Hospital approved this study. All the T2DM patients were defined according to the 1999 World Health Organization (WHO) criteria and randomly recruited from the outpatient department. The controls had a fasting plasma glucose concentration <5.1 mmol/L and HbA1C $<6\%$, with no history of oral hypoglycemic or lipid lowering agents. We collected the medical history and demographic information of all the individuals. Total study population was grouped according to age, gender, insulin, glycemic status and obesity. However, 728 patients were excluded due to other endocrine diseases. The remaining 772 patients were categorized as having $IR > 2$ and $IR < 2$ based on the homeostatic model assessment-estimated insulin resistance (HOMA-IR) index. Statistical analysis was used to examine and link the anthropometric and biochemical profiles with the $IR > 2$ and $IR < 2$ groups. In comparison to the $IR < 2$ group, the total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), and serum insulin levels were considerably higher in all the $IR > 2$ group. Obesity and dyslipidemia were found to be common IR components. According to a generalized linear model, IR was significantly impacted by TC:LDL and TG:HDL. In comparison to age groups I (20–40 years old) and III (61–80 years old), participants in the age group II (41–60 years old) showed considerably higher lipid profiles. These findings provide credence to the idea that lipoprotein ratios may serve as biomarkers for measuring IR. During this study period, novel corona virus affected different people in different regions worldwide. The COVID-19 patients with DM more likely exhibited severe inflammatory response. In an effort to comprehend the connection between COVID-19 and diabetes mellitus and to assess the most affordable treatment option for the general population, the medical data of all suspected patients from 1 May 2020 to 15 August 2020 in the Medical College and Hospital aforementioned were included in the study. A total of 250 suspected COVID-19 patients were considered for this study. Among them, 211 patients were reviewed for laboratory data availability. Most of these patients had mild

symptoms and a good prognosis. All of the 211 patients were subjected to test for COVID-19 confirmation by qRT-PCR. Among them, 98 patients were confirmed COVID-19 positive. Several blood biomarkers in T2DM and non-diabetic (NDM) COVID-19 positive patients were analyzed to rapidly predict COVID-19 progression and severity. In the serum of COVID-19 patients, substantial amounts of ferritin, C-reactive protein (CRP), D-dimer, ALT, and troponin I. In comparison to COVID-19 positive patients without diabetes, the COVID-19 patients with T2DM had increased levels of HbA1C, serum ferritin, and CRP. Data in the present study support the notion that ferritin and HbA1c levels for DM patients, and ferritin, D-dimer, ALT for NDM patients could be biomarkers for progression and severity assessment of COVID-19. However, CRP and Troponin-I could be biomarkers only for poor prognosis of COVID-19. Insulin receptor is a big warehouse of diseases such as T2DM. Any change or mutation in insulin receptor (INSR) may change disease pathogenesis.

Single nucleotide polymorphisms (SNPs) may fall within coding sequences of genes (Non-synonymous), non-coding regions of genes (synonymous), or in the intergenic regions between genes. Non-synonymous SNPs (nsSNPs) may have deleterious effect due to substitution of single amino acids in the protein sequence. The harmful nsSNPs in the INSR gene was analyzed based on various computational approaches. The computational analysis indicated that 13 of these mutations nsSNPs decreased protein stability and may have led to function loss. Two nsSNPs such as I448T and W1220L positions (rs1051691 and rs52800171, respectively) were predicted as "Highly Destabilizing". Their inclusion in the INSR raises the risk of diseases caused by the INSR and altered transcriptional and cell cycle control. In order to search SNPs in the INSR in Bangladeshi subjects, genomic DNA were isolated from healthy individuals and T2DM patient for sequences analysis. Polymerase chain reaction was carried out with primers from different exons of the INSR. Sequence analysis showed that Bangladeshi diabetic patients included in the present study had two mutations in exon 11 of the INSR. However, no mutation was observed in the healthy individuals. The 3D model analysis using bioinformatics tools revealed that the both mutations in the exon 11 may cause conformational change in the INSR. These alterations in the INSR could either slow or speed up the disease's course.

Abbreviations

AGEs	Advanced glycation end products
AKT	Protein Kinase B
ALT	Alanine Aminotransferase
ARDS	Acute respiratory distress syndrome
AUC	Area under curve
BMI	Body Mass Index
COVID-19	Coronavirus disease-2019
CRP	C reactive protein
FBS	Fasting blood sugar
FFA	Free fatty acid
GDM	Gestational diabetes mellitus
GLUT	Glucose transporter
GWAS	Genome-wide association studies
HbA1C	Glycosylated hemoglobin
HDL	High density lipoprotein
HOMA	Homoeostasis model assessment
ICU	Intensive care unit
Ig-A	Immunoglobulin A
Ig-G	Immunoglobulin G
Ig-M	Immunoglobulin M

IM	Intracellular membrane
IRS	Insulin Receptor Substrate
IS	Insulin sensitivity
LDL	Low density lipoprotein
mCSM	Mutation Cutoff Scanning Matrix
NIDDM	Non-insulin-dependent diabetes mellitus
PCOS	Polycystic ovary syndrome
PCR	Polymerase chain reaction
PDB	Protein data bank
RCS	Reactive carbonyl species
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SARS	Severe acute respiratory distress syndrome
SNP	Single nucleotide polymorphism
T ₂ DM	Type 2 diabetes mellitus
TC	Total cholesterol
TNF	Tumor necrosis factor
TG	Triglyceride
VLDL	Very low density lipoprotein
WHO	World Health Organization

Table of Contents

Title	Page
Declaration/Certification	I
Acknowledgement	ii
DEDICATION	iii
Abstract	iv-v
Abbreviations	vi-vii
Table of Contents	viii-x
List of Tables	xi
List of Figures	xii
Chapter 1: Introduction	1-23
1.1 Glucose Homeostasis	1
1.2 Diabetes	2
1.3 Types of diabetes mellitus	3
1.3.1 Type 1 Diabetes Mellitus	4
1.3.2 Type 2 Diabetes Mellitus	4
1.4 Important Causes of T2DM	4
1.5 The Pathophysiology of T2DM	7
1.5.1 Mechanisms of Insulin Resistance	9
1.5.2 Insulin Resistance, Obesity and Dyslipidemia	11
1.5.3 Molecular Basis of Diabetes	15
1.5.4 Response of T2DM to COVID-19	18
1.6 Literature review	20
1.7 Purpose of the study	23
Chapter 2: Relationship among Obesity, Blood Lipids and Insulin Resistance in Bangladeshi Adults	24-39
Summary	24
2.1 Introduction	24
2.2 Materials and Methods	26
2.2.1 Population analysis, grouping, and sample gathering	26
2.2.2 Anthropometric analyses	27
2.2.2.1 Anthropometric measurements	27
2.2.2.2 Measurement of blood pressure	28
2.2.3 Biochemical analyses	28
2.2.4 Insulin sensitivity analyses	28
2.2.4.1 Homoeostasis model assessment (HOMA)	28
2.2.5 Body mass index	29
2.2.6 Blood analyses	29
2.2.6.1 Estimation of serum glucose	29
2.2.6.2 Estimation of Glycosylated Hemoglobin (HbA1c)	29
2.2.6.3 Estimation of lipid profile	30
2.2.6.3.1 Estimation of Total Cholesterol	30
2.2.6.3.2 Estimation of triglycerides	30
2.2.6.3.3 Estimation of high-density lipoprotein (HDL)-cholesterol	30
2.2.6.3.4 Estimation of low-density lipoprotein (LDL)-cholesterol	31
2.2.7.4 Estimation of serum insulin	31

2.2.8 Statistical analyses	31
2.3 Results	31
2.3.1 Anthropometric and biochemical events associated with insulin resistance	31
2.3.2 Lipid phenotypes in insulin resistance	34
2.3.3 Combination of dyslipidemia and insulin resistance in participants of varying ages	35
2.4 Discussion	37
Chapter 3: Blood Biochemical Parameters for Assessment of COVID-19 in Diabetic and Non-Diabetic Subjects	40-55
Summary	40
3.1 Introduction	40
3.2 Materials and Methods	43
3.2.1 Considerations for choosing diabetic patients	43
3.2.2 Exclusion Criteria	43
3.2.3 Questionnaire	43
3.2.4 Collecting and Storing of Blood Samples	44
3.2.5 Anthropometric Measurements	44
3.2.6 Measurement of Blood Pressure	44
3.2.7 Biochemical analysis	44
3.2.7.1 Estimation of serum creatinine	44
3.2.7.2 Estimation of Serum ALT	45
3.2.7.3 Measurement of Troponin I	45
3.2.7.4 Estimation of Serum Ferritin Level	45
3.2.7.5 Quantitative determination of serum CRP by nephelometer	45
3.2.7.6 Estimation of D-dimer level	45
3.3 Statistical analysis	45
3.4 Results and Discussion	46
3.4.1 Baseline data	46
3.4.2 Blood biochemical parameters in COVID-19 positive and negative patients	48
3.4.3 Effects of DM on COVID-19 positive patients	52
3.4.4 Explorative data analysis	54
Chapter 4: Computational Analysis of Damaging Single-Nucleotide Polymorphisms and Their Structural and Functional Impact on the Insulin Receptor	56-73
Summary	56
4.1 Introduction	56
4.2 Materials and Methods	57
4.2.1 Datasets	57
4.2.2 Analysis of Protein Variation Effects	58
4.2.3 3D Modeling and Analysis of Protein Structure	59
4.2.4 Structure Validation and Energy Minimization	59
4.2.5 Protein Stability Validation for Mutant Structure	59
4.2.6 Structural Analysis	60
4.3 Results and Discussion	61
4.3.1 SNP Dataset from dbSNP	61
4.3.2 Effects of nsSNPs on INSR Predicted by Different Tools	63
4.3.3 Effects of nsSNP on Protein Structure	68
4.3.4 Effects of nsSNP on Protein Stability	72

Chapter 5: Analysis of Single Nucleotide Polymorphism in Insulin Receptor in Diabetic Subjects	74-86
Summary	74
5.1 Introduction	74
5.2 Materials and Methods	76
5.2.1 Isolation of DNA from Blood	77
5.2.2 PCR Mediated Amplifications of the Exons in the Insulin Receptor Gene	78
5.2.3 PCR Products Purification and DNA Sequencing	78
5.2.4 Bioinformatic Analysis of DNA Sequence for Prediction of SNPs in INSR	80
5.2.5 Mutation analysis and homology modeling	81
5.3 Results and Discussion	81
5.3.1 Generation of Consensus Sequence	81
5.3.2 Mutation in the INSR of Diabetic Patient	82
5.5 Outcomes of the Research	86
Conclusion	87
References	88-113

List of Tables

Table No.	Table Legend	Page No.
Table 1.1	Prevalence of Diabetes in Asian Countries	3
Table 1.2	Adult BMI classifications	12
Table 2.1	Subject characteristics subdivided by insulin status	32
Table 2.2	Subjects characteristics by obesity category	33
Table 2.3	Analysis of multiple regression of lipid status and lipid ratio with the total number of individuals with insulin resistance	34
Table 2.4	Generalized linear model for persons with diabetes (FBS>7) and without diabetes (FBS<7)	35
Table 2.5	Multiple comparisons using Tukey's analysis of the mean BMI with respect to several age groups	36
Table 3.1	Baseline parameters of patients with and without COVID-19 (mean values of several factors)	48
Table 3.2	COVID-19 negative and COVID-19 positive patients' biochemical findings	49
Table 3.3	Biochemical parameters of patients with DM and Non-DM	52
Table 4.1	List of nsSNPs that were predicted to have functional significance by PROVEAN	62
Table 4.2	Potential effect of amino acid substitution for nsSNPs in human INSR gene predicted by the PolyPhen algorithm	64
Table 4.3	List of nsSNPs stability predicted by I-MUTANT	66
Table 4.4	Common amino acid change of deleterious nsSNPs in human INSR gene predicted by PROVEAN and PolyPhen algorithms.	67
Table 4.5	Mapping of nsSNPs in 2HR7 and 4IBM 3D structures	68
Table 4.6	RMSD and total energy after energy minimization of native structures and their mutant 3D models	70
Table 4.7	Protein stability upon mutation	72
Table 5.1	Pairs of primers for amplifying exons of the human insulin receptor gene	78

List of figures

Figure No.	Figure Legend	Page No.
Figure 1.1	Mechanism of glucose-stimulated insulin secretion	2
Figure 1.2	The natural history of type 2 diabetes	8
Figure 1.3	The diagnostic criteria of diabetes.	8
Figure 1.4	Insulin increases or decreases a variety of intracellular metabolic pathways by acting through its receptor to influence several physiological processes in the organism	11
Figure 1.5	Significant physiological functions of insulin in the skeletal muscles, liver, and adipose tissue	14
Figure 1.6	Modular structure of the insulin receptor	16
Figure 1.7	Each targeted protein contains an active site	18
Figure 1.8	Potential pathogenic events in COVID-19 patients with T2DM	19
Figure 2.1	Grouping of population of interest for the research	27
Figure 2.2	Percentage of insulin resistance based on age group	36
Figure 2.3	Lipid profiles of insulin-resistant individuals of three different ages	37
Figure 3.1	Reasons of diabetes with COVID-19 patients	42
Figure 3.2	Work flow of the study	47
Figure 3.3	Ferritin boxplots in DM and NDM patients with positive and negative COVID-19 findings	54
Figure 3.4(a)	3D plot of Ferritin, D-dimer and CRP as these variables might play role in detecting COVID and non-COVID	55
Figure 3.4 (b)	Use principal component analysis of variables Ferritin, CRP and d-dimer for partitioning the groups	55
Figure 4.1	A flow chart of bioinformatics tools	61
Figure 4.2	A comparison of amino acid substitutions due to nsSNPs	69
Figure 4.3	Superimposition of native and mutant structures.	71
Figure 5.1	A flow chart of methodologies used in the present progress report.	77
Figure 5.2	DNA bands of exon 6 after gel purification. Lane M: Molecular weight marker (1 kb DNA ladder, Promega Corporation); Lanes 1 & 2 show the purified PCR product of exon 6.	79
Figure 5.3	DNA bands of exon 11 after gel purification. First lane: Molecular weight marker (1 kb DNA ladder, Promega Corporation); the indicated bands represent the purified PCR product of exon 11.	79
Figure 5.4	DNA bands of exon 20 after gel purification. Lane M: Molecular weight marker (1 kb DNA ladder, Promega Corporation); Lanes 1 & 2 show the purified PCR product of exon 20.	80
Figure 5.5	DNA bands of exon 21 after gel purification. Lane M: Molecular weight marker (1 kb DNA ladder, Promega Corporation); Lanes 1 & 2 show the purified PCR product of exon 21.	80
Figure 5.6	BLASTn results of the consensus sequence generated from exon 11	82
Figure 5.7	Three mutations found threonine to proline, phenylalanine to serine, glutamate to proline	83
Figure 5.8(a)	Homology modeling of insulin receptor	84
Figure 5.8(b)	3D structure analysis of the insulin. The Swiss-model was used to construct 3D structure, and these structures were visualized via PyMol.	84
Figure 5.9	The structural conformation of the INSR	85

CHAPTER ONE

Introduction

1.1 Glucose Homeostasis

Insulin, a hormone made up of 51 amino acids, plays a crucial function in glucose homeostasis. Hyperglycemia is a condition characterized by elevated glucose levels in the bloodstream. A healthy person's plasma glucose level is kept between 70 and 165 mg/dl throughout the day by a number of physiological regulatory mechanisms. Below a certain level of plasma glucose, blood flow to the brain is restricted, which triggers the production of glucagon, adrenalin, and cortisol and raises the plasma glucose concentration. If plasma glucose concentration is over the optimum range, the hypothalamus acts on the cells to release insulin, and the normoglycemic condition is maintained by stimulating glucose transport, utilization and storage (de Lemos *et al.*, 2012). Glucose is the main source of fuel for the cells in our body, and by facilitating glucose clearance into skeletal muscle and to a lesser extent in the liver and adipose tissues, insulin regulates glucose homeostasis. When insulin interacts to cell surface receptors, vesicles containing the glucose transporter (GLUT-4) migrate to the plasma membrane and fuse through endocytosis, thereby facilitating the diffusion of glucose into the cell (Berger & Zdzienko, 2020). High levels of glucose in the blood (hyperglycemia) due to hormonal and metabolic disorders cause many complications. Type 1 diabetes mellitus is caused by insufficient or non-existent production of insulin. Type 2 diabetes is primarily due to a decreased response to insulin in the tissues of the body (Czech, 2017). Tyrosine kinase receptors, such as the insulin receptor (INSR), function by adding a phosphate group to specific tyrosine on specific proteins inside of a cell. A protein known as insulin receptor substrate 1 (IRS-1) is one of the substrate proteins that the insulin receptor phosphorylates. Because of IRS-1 binding and phosphorylation, which results in an increase in high affinity glucose transporter type 4 (GLUT-4) molecules on the outer membrane of insulin-responsive tissues including muscle cells and adipose tissue, the absorption of glucose from the blood into these tissues rises. In other words, the GLUT-4 is transferred from the cell surface to the cellular vesicles, where it can enhance glucose transport into the cell (Fig.1.1).

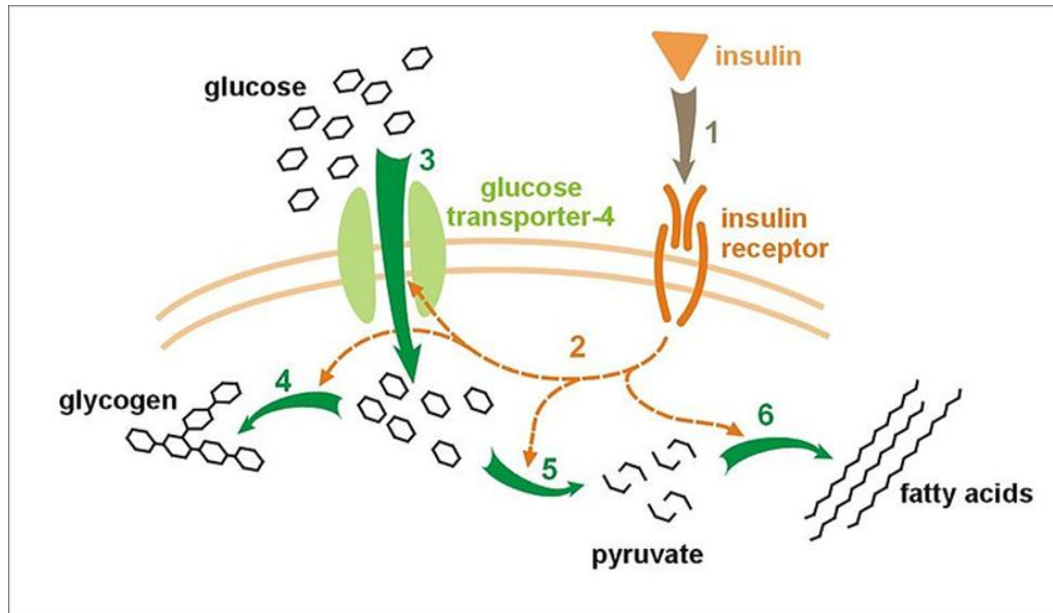


Figure 1.1: Mechanism of glucose-stimulated insulin secretion. Insulin binds to its receptor (1) starts numerous protein activation cascades (2), and cause translocation of GLUT-4 transporter to the plasma membrane for influx of glucose (3), glycogen synthesis (4), glycolysis (5) and fatty acid synthesis (6). The figure was adopted from Meiquer, (2006).

1.2 Diabetes

Hyperglycemia developed by hormonal and metabolic disorders cause many complications. Diabetes mellitus (DM) is one of the important complications generated by hyperglycemia. The terminology “Diabetes mellitus” refers to a group of metabolic diseases that are carried on by deficiencies in insulin production, activity, or both. Long-lasting high blood sugar levels and abnormalities in protein, fat, and carbohydrate metabolism due to deficiencies in insulin action, secretion, or both are characteristics of DM. The World Health Organization (WHO) published the first global report on diabetes in April 2016. To highlight the state of diabetes prevention and control in each WHO Member State, WHO created a series of diabetes country profiles to go along with this report shown in Table 1.1 (WHO, 2016). DM is a complicated disorder caused by a combination of lifestyle variables like excessive weight and lack of physical activity as well as genetic abnormalities including monogenic and polygenic mutations. Type 2 DM (T2DM) is brought on by relative insulin insufficiency or diminished biological effects of insulin. About 90–95% of all diabetic disorders worldwide are caused by non-insulin-

dependent diabetes mellitus (NIDDM), often known as type 2 diabetes. It is also among the most common kinds of diabetes. Either insufficient insulin release or inactive insulin causes this serious condition (Taguchi & White, 2008). It occurs at the age of 40. Because T2DM is a worldwide issue, it should be given more priority to be addressed, and the health care delivery system should implement beneficial policies (Avogaro *et al.*, 2011). Numerous problems, including macrovascular and microvascular abnormalities, are brought on by DM (Hudda *et al.*, 2019). Furthermore, T2DM treated as a polygenic group of disorders is developed due to insulin resistance (IR), β -cell dysfunctions, or both, and the molecular basis of T2DM has been characterized (Berger & Zdziebło, 2020). It is currently understood that T2DM patients have polygenic medical traits, which are brought on by hereditary factors (Miao *et al.*, 2010).

Table 1.1 Prevalence of Diabetes in Asian Countries (WHO 2016)

Country	Percentage of diabetic patients (Aged 20-79 years)
Bangladesh	8.3
China	9.8
India	9.3
Indonesia	6.5
Korea	4.4
Malaysia	17.9
Nepal	3.7
Pakistan	8.1
Sri Lanka	8.0
Thailand	7.1
Vietnam	6.0

Collected from WHO, 2016

1.3 Types of Diabetes Mellitus

Type 1 diabetes, also known as insulin-dependent diabetes, and type 2 diabetes, also known as non-insulin-dependent diabetes mellitus, are the two basic categories under which DM is categorized. The self-destruction of pancreatic β -cells, which results in a subsequent insulin deficit, is what makes Type 1 diabetes mellitus (T1DM) unique. IR and insulin insensitivity (IS) are the causes of T2DM. In certain circumstances, specific mutations result in genetic susceptibilities such hereditary impairments of pancreatic β -cell activity or anomalies in insulin action. Additionally, gestational diabetes, a kind of diabetic illness, primarily affects pregnant women (De Marinis *et al.*, 2010).

1.3.1 Type 1 Diabetes Mellitus

T1DM is a serious condition where blood glucose level is too high because body can't make insulin. Different factors, such as genetics and some viruses, may cause type 1 diabetes (IDF, 2011). T1DM is an autoimmune or self-destructive condition. However, under this self-destructive illness, body's own tissues are destroyed by its native defense mechanism (Tao *et al.*, 2015). Usually, T1DM also known as juvenile diabetes is developed in youth and teenagers (Busik *et al.*, 2009). It is also called insulin-dependent diabetes mellitus (IDDM) (Tomic *et al.*, 2022).

1.3.2 Type 2 Diabetes Mellitus

High blood glucose levels are a hallmark of T2DM and are caused by irregular insulin release from the pancreatic β -cell, IR in peripheral tissues, and accelerated glycogenolysis (Png *et al.*, 2016). T2DM is far more prevalent than T1DM; it often develops after the age of 30, and usually between the ages of 50 and 60. T2DM proceeds gradually. As a result, the term "adult-onset diabetes" is frequently used to describe this illness. However, the number of persons with T2DM who are younger than 30 years old is rising daily (Samuel & Shulman, 2012). T2DM is frequently correlated with obesity, inactivity, and poor eating habits. Important T2DM therapy options include diet and exercise modifications alone, oral medications, and even insulin injections (Imierska *et al.*, 2020). Predisposition to T2DM may partly be due to mutations in the INSR (Pinzi & Rastelli, 2019).

1.4 Important Causes of T2DM

(i) Genetic Vulnerability

Genetic predisposition is a significant factor in T2DM susceptibility. However, specific genes or gene combinations may increase or decrease a person's risk of contracting the disease. High rates of T2DM in twins, and significant racial disparities in the prevalence of diabetes, suggest the involvement of genes with this serious complication (Triolo *et al.*, 2019).

(ii) Sedentary Lifestyle

Sedentary behavior and being overweight are strongly associated with T2DM. Insulin inactivity is brought on by obesity in grade 1. People with T2DM tend to be overweight more frequently. It could result from an imbalance between energy intake and inactivity. Only people who are centrally obese- those who have too much belly fat been at high risk for developing IR. A greater body mass index (BMI) is another sign of T2DM (Nagao *et*

al., 2015). Calorie restriction is linked to a longer lifespan in diabetic person (Napoleao, 2021)

(iii) Insulin Resistance

IR develops in people having overweight or obese, lots of belly fat, and no frequent exercise (Lumeng *et al.*, 2007). Additionally, IR happens due to lack of response of muscle, fat, and liver cells to insulin as they ought to, causing the pancreas to overproduce insulin as a means of regaining balance. A few of the reasons that cause IR include high glucocorticoid levels, elevated growth hormone, gestational diabetes mellitus (GDM), polycystic ovarian syndrome, INSR autoantibodies, and hemochromatosis (Mather *et al.*, 2001).

(iv) Fault in Insulin Release

A modification to pancreatic β -cells' structure associated with hyperglycemia is thought to be the etiology of T2DM and IR. In response to IR, β -cells attempt to produce more insulin to tolerate glycemia. Increased insulin production is associated with larger islets and a higher proportion of β -cells (Azulay *et al.*, 2022). The outcome is, β -cells impair insulin secretion. Additionally, intracellular triglyceride accumulation might result in β -cell dysfunction (Tang *et al.*, 2009).

(v) Glucagon

Blood sugar levels must be maintained by α -cell production of pancreatic glucagon. This hormone affects insulin in the opposite manner. Reduced blood sugar levels stimulate the secretion of α -cell (Liu *et al.*, 2012). In diabetes situations, insulin release is inadequate at hyperglycemic condition and is not inhibited at higher glucose levels (Reed *et al.*, 2020).

(vi) Oxidative Stress

The reactive oxygen species (ROS) such as superoxide, hydroperoxyl, and nitric oxide modify the cell membrane (Diz-Chaves *et al.*, 2020). These ROS result in increased blood glucose and free fatty acid (FFA) levels, which aid in the development of T2DM and IR. Diabetes worsens as a result of rising ROS generation and declining antioxidant enzyme activity (Yaribeygi *et al.*, 2020).

(vii) Polycystic Ovary Syndrome (PCOS)

IR and ovarian androgen production are clearly increased in association with PCOS. PCOS affects approximately 6–10% of all women and is a prevalent endocrine condition that affects women regularly (Xu & Qiao, 2022).

(viii) Excessive Glucocorticoid Production

The Cushing's syndrome, which results in excessive glucocorticoid hormone production, reduces various tissues' sensitivity to insulin's metabolic properties. Diabetes mellitus develops as a result of it (Hill *et al.*, 2021).

(ix) Production of Unusual amount of Glucose in the Liver

When glucagon levels rise, the liver produces too much glucose, which raises blood sugar levels (Chadt & Al-Hasani, 2020).

(x) Genetic Diseases

T2DM is associated with genetic diseases such hemochromatosis and cystic fibrosis. Diabetes is brought on by cystic fibrosis, which also inhibits the pancreas and produces unusually thick mucus (Berger & Zdzieblo, 2020). Destruction of the pancreas or its removal pancreatic trauma, malignancy, and improper insulin production are all connected with the development of T2DM (Haeusler *et al.*, 2018).

(xi) Medications

Medicines, such as nicotinic acid and various diuretic kinds, pharmaceuticals that prevent seizures, and antipsychotic medications, can decrease insulin function. Diabetes, β -cell dysfunction, and pancreatitis are all risks if that medication can increase. The impact of arsenic on the development of diabetes is identical (Di Pino & De Fronzo, 2019).

(xii) Chemical Toxins

Increased risk of diabetes is associated with a high intake of substances that contain nitrogen, such as nitrates and nitrites. Arsenic has also been researched for possible links to diabetes development (Sarmiento *et al.*, 2019).

(xiii) Lipid Breakdown

Lipodystrophy is brought on by a reduction of fat content inside the body. Lipodystrophy is linked to T2DM and insulin inactivity (Trouwborst *et al.*, 2018).

(xiv) Role of SNPs on Diabetes Mellitus

Insulin, insulin growth factor I, and insulin growth factor II activate the INSR, a tyrosine kinase-specific transmembrane receptor (Belfiore *et al.*, 2017). Controlling glucose homeostasis, which can lead to a range of clinical consequences like diabetes and cancer, is a major metabolic function of the INSR (White & Kahn, 2021). The major function of the INSR, stimulating glucose uptake, is compromised by a reduction in insulin receptor signaling, a contributing factor to T2DM. The cells' failure to absorb glucose results in diabetes and all of hyperglycemia's negative effects. It has been established that the

presence of mutant receptors within the cell may negatively impact the function of the normal receptor (Albegali *et al.*, 2019). An earlier study using INSRs transfected into cultured cells discovered that the function of native INSRs was diminished by kinase-deficient INSRs, which also acted as dominant-negative mutations (Marucci *et al.*, 2009). Generally, the mutation of INSR results as a recessive form (Kasuga, 2019). The function and implications of the recessive mutation in the INSR were examined. The majority of human genetic variations, or almost 500,000 single-nucleotide polymorphisms (SNPs), are found in the exons of the genome (Sortica *et al.*, 2015). Nonsynonymous SNPs (nsSNPs), a subclass of these SNPs, have the power to change the amino acid residues and increase the variety of activities of encoded proteins in the human population. It is clear that there are variances in the SNPs' chromosomal distribution. SNPs frequently appear in non-coding regions (Oo *et al.*, 2013). SNP density can also be impacted by genetic recombination and mutation rate (Kasuga *et al.*, 1982). Due to the fact that SNPs are often biallelic, one SNP can produce a Mendelian disease (Kahn & Folli, 1993). Some SNPs are crucial pharmacogenomic targets for drug discovery since they are also connected to the metabolism of a variety of medications (Blazquez *et al.*, 2014). SNPs can act as a helpful genetic marker for genome-wide association studies (Mei *et al.*, 2016). The implications or negative effects of SNPs are frequently linked to how they alter the composition and biological activity of proteins. But only a very tiny number of researches have looked at the SNPs and their impact on INSR.

1.5 Pathophysiology of T2DM

T2DM is a chronic metabolic disease characterized by an abnormal increase in plasma glucose (Henry, 1998) shown in Figure 1.2. We now know that these diagnostic criteria shown in Figure 1.3 focus on only a small part of the disease process (Salber *et al.*, 2008). The diagnosis criteria for diabetes outlined by the American Diabetes Association are as follows-(i) Fasting plasma glucose greater than 126 mg/dL, (ii) a random plasma glucose higher than 200 mg/dL in association with symptoms of diabetes, and/or (iii) a persistent elevation in plasma glucose following an oral glucose load (greater than 200 mg/dL two hours after glucose ingestion (WHO, 2017). HbA1c 6.5% may be considered as a cut-off for diabetes, HbA1c 5.9% is ideal for diagnosing prediabetes, and value 5.6% eliminates prediabetes/diabetes condition, according to research to evaluate the diagnostic accuracy

and best HbA1c cutoffs for diabetes and prediabetes among high-risk south Indians (Radhakrishna *et al.*, 2018).

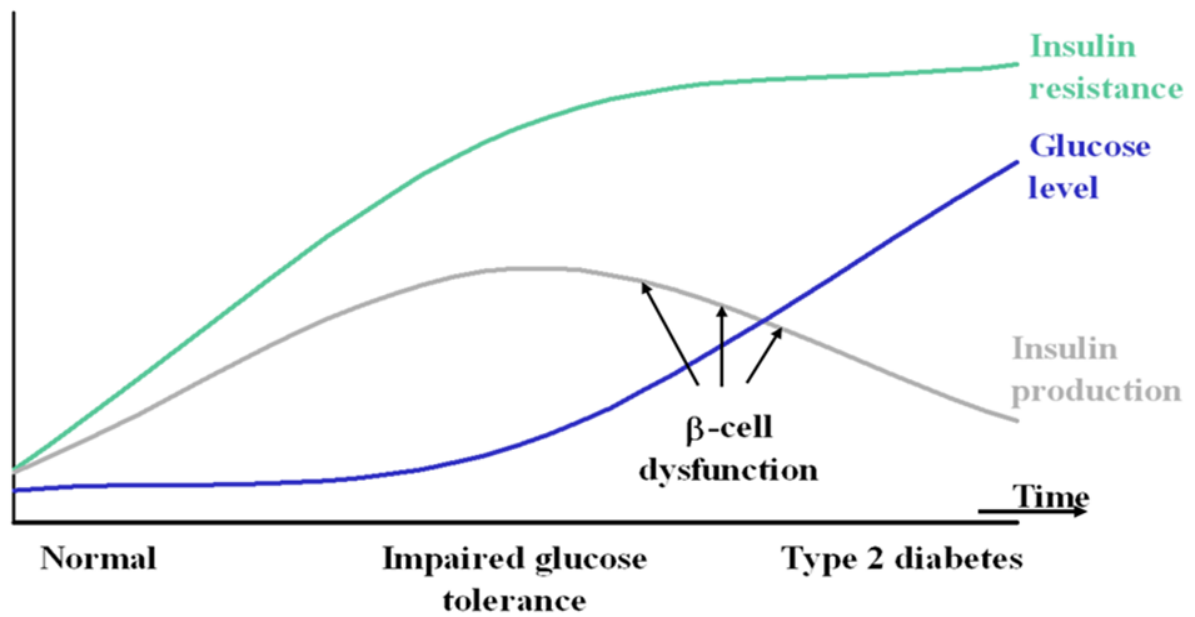


Figure 1.2: The natural history of type 2 diabetes Adopted from Henry, (1998).

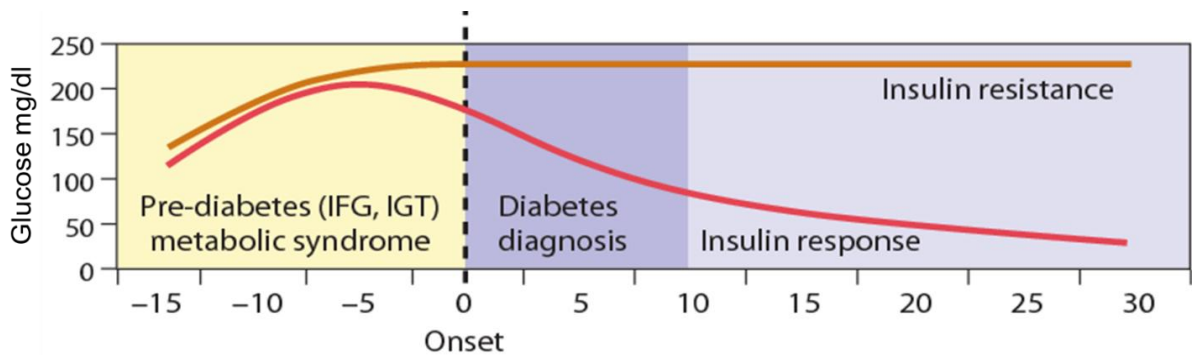


Figure 1.3: The diagnostic criteria of diabetes. It's usual to have blood sugar levels under 140 mg/dL (7.8 mmol/L). After two hours, a result of blood sugar of greater than 200 mg/dL (11.1 mmol/L) indicates diabetes. Prediabetes is identified by a value between 140 and 199 mg/dL (7.8 mmol/L and 11.0 mmol/L). Collected from Salber *et al.*, (2008).

1.5.1 Mechanisms of Insulin Resistance

Biology and medicine both focus on the mechanism of insulin action. IR, which plays a significant role in the development of atherosclerotic process, is a constellation of biochemical and physiological derangements of metabolic abnormalities, including (i) hyperinsulinemia, (ii) glucose intolerance, (iii) increased low density lipoprotein (LDL), (iv) decreased high density lipoprotein (HDL), and (v) hypertension. Depending on the pathophysiologic condition, there are probably multiple etiologies for the complicated metabolic abnormality known as IR (Haeusler, McGraw, & Accili, 2018). In summary, IR is a complicated trait that includes communication between several organs, such as the pancreas, liver, and muscle. Genetic flaws, fat-derived signal (ectopic lipid buildup), inactivity, obesity, and inflammation can all contribute to IR (Kalupahana *et al.*, 2012; Samuel & Shulman, 2012). The main focus of research has been to investigate the role of insulin in the onset and progression of pathological conditions and chronic diseases, such as diabetes. It has been proposed that hereditary abnormalities in mitochondrial function with a reduced capacity to oxidize fatty acids are contributing factors in individuals impaired IS in T2DM patients (Semiz *et al.*, 2013). Among its many roles as a pleiotropic hormone are the stimulation of nutrient uptake into cells, control of gene expression, alteration of enzyme activity, and maintenance of homeostasis in terms of energy (Rabiee *et al.*, 2018). The phosphorylation and dephosphorylation of serine and/or threonine residues appears to control the enzymes involved in insulin's regulation of glucose metabolism (Scapin *et al.*, 2018). Alterations in glycogen metabolism, which affect phosphoenol pyruvate carboxykinase, the rate-limiting enzyme in gluconeogenesis, can also affect glucose hemostasis (Tan *et al.*, 2015). In skeletal muscle, several mitochondrial enzymes activities decrease 20-40% in insulin-resistant individuals (Pinti *et al.*, 2019). Recent developments in insulin research have allowed for the development of insulin-signaling activators and targeted therapeutics for various diseases. Genetic and environmental variables, especially those linked to obesity, affect IS. Hyperinsulinemia is brought on by increased IR, whether it might be hereditary or environmental (Inokuchi *et al.*, 2008). As muscle's ability to use glucose declines, it is sent to the liver, where it is converted to fat and stored there. Insulin encourages the formation of fat in the liver during a favorable energy balance. If more calories are consumed, a person who has some degree of muscular IR will build liver fat more quickly than other people (Mather *et al.*, 2001).

Four things happen as the amount of liver fat increases: 1) hepatic IR, 2) an increase in hepatic glucose production because the liver is less sensitive to insulin's ability to suppress it, 3) an increase in plasma glucose that worsens hyperinsulinemia, and 4) further hepatic fat deposition that is exacerbated by elevated plasma glucose and portal hyperinsulinaemia (Youngren, 2007). When the liver's fat content rises, more triacylglycerols (TGs), which accumulate in ectopic locations such as pancreatic islets, are secreted into the blood. Ectopic fat reduces the amount of insulin that cells produce in response to consumed glucose, which causes plasma glucose to increase even more. These behaviors ultimately lead to the development of clinical diabetes by restricting insulin release from beta cells and promoting cell death (Potenza *et al.*, 2009). In summary, IR is a complicated trait that includes communication between several organs, such as the liver, pancreas and muscle.

Insulin receptor substrate (IRS) is activated by phosphorylation. IRS-2 is primarily found in the cytoplasm, while IRS-1 is a transmembrane protein. IRS-1 serves as an essential component of the insulin secretion processes in skeletal muscle. The growth of insulin-producing cells in the liver and pancreas is regulated by IRS-2. Both IRS-1 and IRS-2 undergo fast tyrosine phosphorylation in response to insulin stimulation. Despite the fact that IRS-2 is primarily found in the cytosol, most of this takes place in the intracellular membrane (IM) compartment (Belfiore *et al.*, 2017). Tyrosine phosphorylation of IRS is necessary for insulin responsiveness, although IRS can either increase or decrease insulin activity depending on which serine is phosphorylated (Ormazabal *et al.*, 2018). In order to activate the tyrosine kinase in the α -subunit of the receptor, insulin first must connect to the receptor's β -subunit. Additionally, this mechanism initiates the autophosphorylation of a number of tyrosine residues found in the β -subunit (Bhardwaj *et al.*, 2011). The IRS protein cascades are the basic components of the peripheral response because they are recognized by other members of the signaling pathway for further downstream action. Finally, it results in the body absorbing and storing glucose as glycogen (Zheng *et al.*, 2017). As a result, the pancreatic β -cell development and function, as well as signaling, are all influenced by the insulin receptor substrate family (Softic *et al.*, 2017). Insulin prevents gluconeogenesis in the liver, hence IR in the liver results in increased hepatic glucose synthesis. By reducing hormone sensitive lipase activity, insulin signaling in adipocytes reduces lipolysis. This anti-lipolytic impact also prevents free fatty acid efflux from adipocytes to the liver. Decreased IS in skeletal muscle and inhibition of hepatic glucose

synthesis are caused by an increase in free fatty acids in the blood (Berger & Z dzieblo, 2020). A unique family of lipids produced by adipose tissue cannot be made when GLUT-4 and lipogenesis in adipocytes are reduced. Insulin signaling promotes triacylglycerol production and inhibits lipolysis, which together increase lipid storage in adipocytes (Miao *et al.*, 2020). Nitric oxide generation from endothelial cells is reduced by IR, and the release of pro-coagulant substances that trigger platelet aggregation is increased (Choi *et al.*, 2010). In adipose tissue, insulin promotes glucose uptake via a GLUT-4 dependent mechanism. Adipose tissue utilizes glycolysis for energy purposes. Insulin promotes re-esterification of fatty acids into triglycerides and enhancing the pool of glucose-3-phosphate required for esterification. Due to its hydrophilic nature, glucose cannot freely diffuse into or out of a cell. To get across the cell membrane, it requires transporters (Wang *et al.*, 2020). Anabolic peptide hormone insulin is secreted by the β -cells of the pancreas and is essential for controlling human metabolism as shown in Figure 1.4 (De Meyts, 2016).

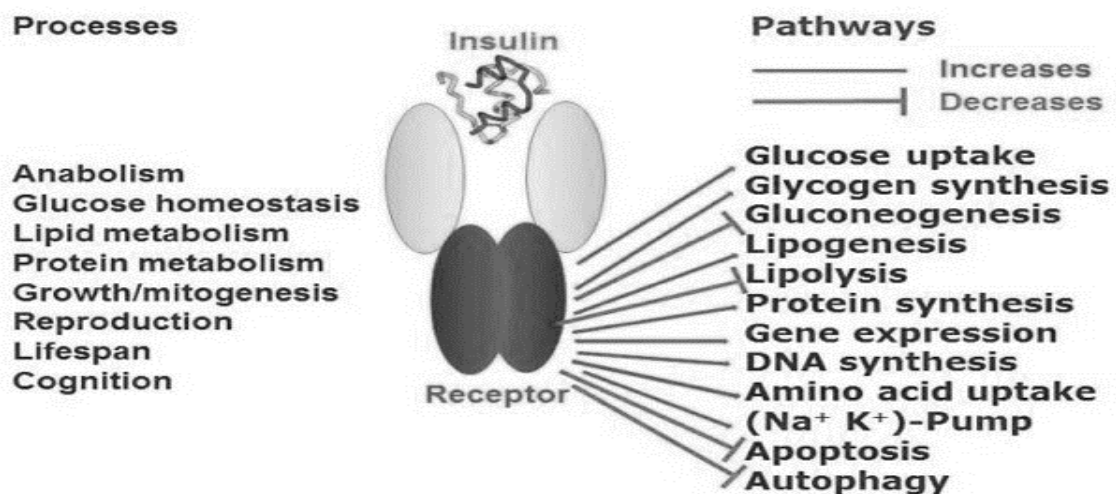


Figure 1.4: Insulin increases or decreases a variety of intracellular metabolic pathways by acting through its receptor to influence several physiological processes in the organism. Adapted from De Meyts, (2016).

1.5.2 Insulin Resistance, Obesity and Dyslipidemia

Metabolic syndrome is a crucial factor for causing T2DM and obesity in South Asians (Png *et al.*, 2016). The metabolic syndrome is regularly present in 20–25% of metropolitan South Asians (Kazemina *et al.*, 2020). Effectively implementing and strengthening

diagnostic standards is crucial for preventing the "epidemic" of obesity and the metabolic syndrome (Sramek *et al.*, 2021). South Asians typically have poor muscle mass, a high percentage of body fat, abdominal obesity, IR, and hyperinsulinemia. Particularly, South Asians frequently have abdominal obesity, which is associated with higher overall IR and impaired endothelial function in this group (Bhardwaj *et al.*, 2011). However, due to South Asian individuals' lower BMI threshold, awareness is growing to South Asian (Herman & Zimmet, 2012). Additional intermediate cut-off values of 23.0 kg/m² and 27.5 kg/m² suggested for increased risk and greater risk, respectively, are proposed in this demographic group since South Asian individuals are more susceptible to obesity-related disorders (Nagao *et al.*, 2013). The fundamental benefit of BMI is that it simply requires height and weight to calculate, and the same cut-off may be used for people of all ages and genders. BMI only assigns people to three categories: normal, overweight, or obese, which can be misleading, especially for athletes and bodybuilders who have high lean body mass. Similar to how women and older persons are more likely to have higher levels of body fat than males and younger people despite having the same BMI. However, population-level misclassifications are not prevalent as BMI correlates well with the population's direct assessment of body fat. Similar to males and young people, women and the elderly may have the same BMI but are likely to have greater body fat; therefore, it is still widely employed in research and at the community level (Henning, 2021).

Table 1.2 Adult BMI classifications

Classification	BMI range (kg/m ²)
Underweight	<18.5
Normal-weight	18.5 to 24.9
Overweight	25 to 29.9
Obese	≥30
Obese Class I	30-34.9
Obese Class II	35-39.9
Obese Class III	≥40

Collected from Henning, (2021)

IR is characterized by insulin exerting a biological effect that is less than anticipated. This syndrome is caused by significant limitations in glycogenesis and, a lesser degree,

glycolysis during the insulin-stimulated glucose uptake (Petersen & Shulman, 2018). Obesity is the outcome of metabolic disruptions that induce tissue stress and malfunction as well as an upset in the energy balance that results in weight increase. Increased adiposity and inadequate physical activity are strong and independent predictors of IR. Obesity is a global epidemic and strongly linked to the development of T2DM. Increased body weight is also a strong independent predictor of IR in healthy adults (Radenkovic *et al.*, 2011). A lack of knowledge can result in unhealthy eating habits, obesity, insufficient physical activity, and increased psychological stress (Veghari *et al.*, 2013). Obesity greatly influences the lipoprotein composition and variables linked to endothelial dysfunction, inflammatory processes, and vascular injury (Yadav *et al.*, 2017). These modifications might result in increased basal lipolysis in obese people and accompanying changes coupled with metabolic abnormalities that cause lipotoxicity and glucotoxicity contribute to insulin resistant state (Heilbronn *et al.*, 2005, Kahn & Flier, 2000). The targets of circulating insulin, skeletal muscle cells and adipocytes, absorb glucose, bringing blood sugar levels back to normal. Precise role of carbohydrate, protein and lipid metabolism is described in Figure 1.5 (Rahman *et al.*, 2021).

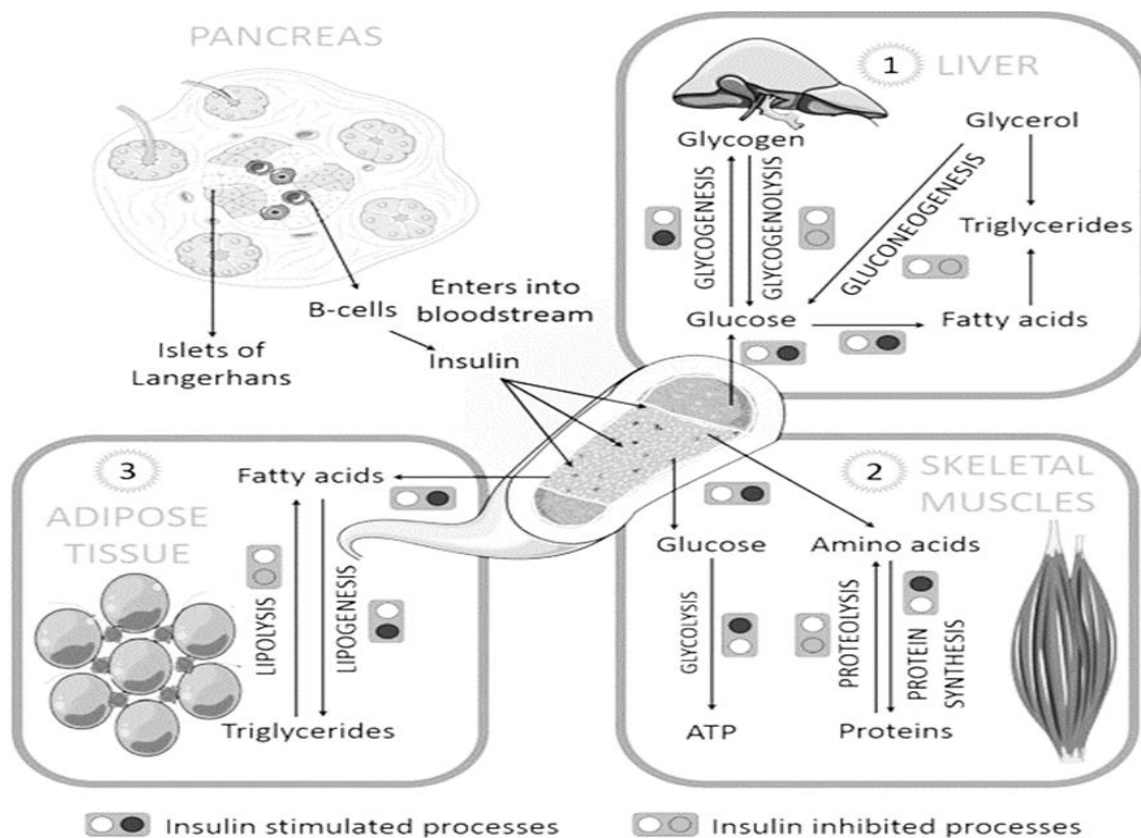


Figure 1.5: Significant physiological functions of insulin in the skeletal muscles, liver, and adipose tissue. In liver and skeletal muscle, insulin increases uptake of glucose and stimulates glycogenesis but decreases gluconeogenesis and lipolysis (1, 2). In adipose tissue, insulin stimulates lipogenesis and inhibits lipolysis (3) Adopted from Rahman *et al.*, (2021). Patients with IR have a unique dyslipidemia that includes hypertriglyceridemia, high apolipoprotein B blood levels, tiny, dense LDL cholesterol, and low HDL cholesterol levels (Kaur *et al.*, 2018). Ceramides are sphingolipids that are a crucial component of cell membranes. During times of elevated free fatty acids (FFA), their buildup in the liver and muscle may contribute to IR (Tan *et al.*, 2015). Diacylglycerol (DAG), a lipid molecule consisting of two fatty acid chains, has been found to be elevated in several models of IR (Choi *et al.*, 2007). Glucotoxicity acts by increasing oxidative stress, formation of

advanced glycation end products (AGEs), and flux through the hexosamine biosynthetic pathway (Samuel & Shulman, 2012). Through oxidative phosphorylation, chronically elevated glucose levels boost glucose metabolism. This results in reactive oxygen species generation and mitochondrial dysfunction (ROS). Tetrahydrobiopterin levels within cells are decreased due to ROS, which also increases the production of superoxide by eNOS (Musicki *et al.*, 2005). In skeletal muscle, glucosamine, a byproduct of the hexosamine biosynthetic pathway, reduces the absorption of glucose induced by insulin (Chang-Chen *et al.*, 2008). Advanced glycation end products (AGEs) are produced as a result of the overactivation of the hexosamine biosynthesis pathway, which in turn promotes the creation of ROS (Gao *et al.*, 2021). Studies have shown that the lack of ROS-detoxifying enzymes in β -cells limits their ability to protect themselves from excessive ROS generation. The production of ROS is known to eventually activate stress-induced pathways (Yaribeygi *et al.*, 2020). When carbohydrates, lipids, and amino acids are oxidized, reactive carbonyl species (RCS) are created. RCS, ROS and nitrogen species (RNS) are now understood to be crucial transducers in biological systems and to be increased in tissues with diabetes (Jezek, 2019). Increased oxidative stress reduces NO availability, impairs Akt and eNOS activation, and increases IR (Ghosh *et al.*, 2017). Adipose tissue, the liver, the pancreas, and the vasculature are tissues where metabolic abnormalities cause immunological activation, and people frequently have elevated plasma markers of chronic low-grade inflammation (Van den Oever *et al.*, 2010). Significant quantities of TNF- α prevent GLUT-4 from moving to the surface of skeletal muscle cells, preventing insulin-mediated food absorption. Additionally, pro-inflammatory mediators prevent the synthesis of adipokines that reduce inflammation and increase IS, including adiponectin (Yanai & Yoshida, 2019). Insulin-signaling activators are now being employed as preventative strategies against a variety of diseases as a result of recent developments in insulin research.

1.5.3 Molecular Basis of Diabetes

Predisposition to T2DM may partly be due to mutations in the insulin receptor (Pinzi & Rastelli, 2019). Patients with genetic types of severe IR have at least 25 point mutations in the insulin receptor gene (Oo *et al.*, 2013). The insulin receptor has a modular structure encoded by a gene (located on chromosome 19) with 22 exons and 21 introns (De Meyts & Whittaker, 2002) shown in Figure 1.6.

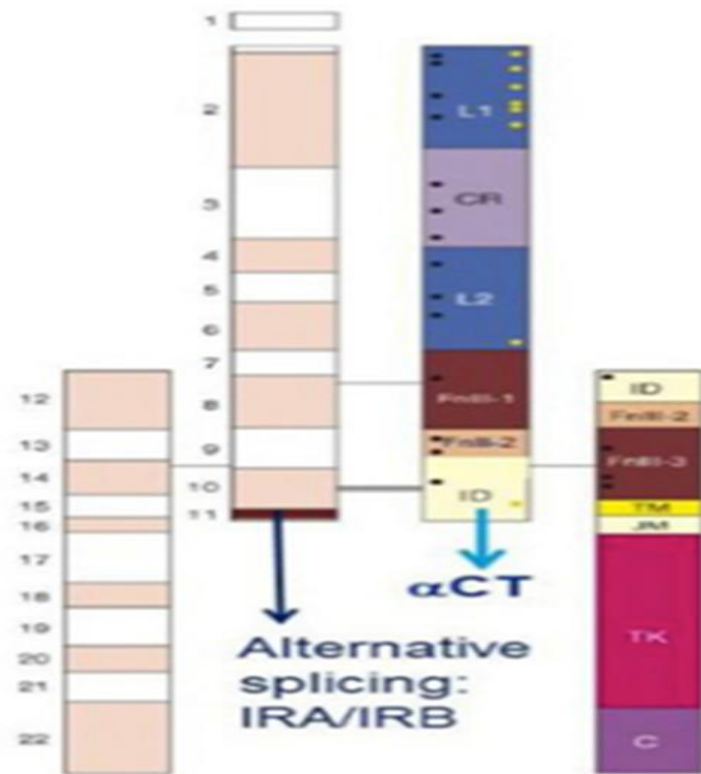


Figure 1.6: Modular structure of the insulin receptor. Exon 11 is highlighted with brown color. Collected from De Meyts & Whittaker, (2002).

By using patient genomic DNA as templates for PCR amplification of each of the 22 exons of the insulin receptor gene, several of these mutations have been discovered through direct sequencing of PCR products (Moller *et al.*, 1990). IR is a complex variable that is impacted by inherited genetic variation, environmental risk factors, and lifestyle risk factors. While epigenetics takes into account extra modifications to the genome that can be passed on to daughter cells independently of changes in the DNA sequence, genetic variation only refers to changes in the DNA code (Maude *et al.*, 2021). Single nucleotide polymorphism (SNPs) can act as a helpful genetic marker for genome-wide association studies. SNPs' repercussions or harmful effects are typically linked to how they affect the structure and function of proteins. Knowledge of *in silico* analysis of SNPs is crucial for comprehending the genetic basis of several complex hereditary human disorders (Mahmud *et al.*, 2016). Absolute confirmation can be achieved using functional and DNA analysis, although certain mutations do not affect insulin binding, and DNA analysis is still unable to detect all suspected mutations. Although there is no direct link between genotype and phenotype, mutations in the insulin receptor's α -subunit are linked to a more severe phenotype than mutations in the β -

subunit. Absolute confirmation can be achieved using functional and DNA analysis, but some mutations do not affect insulin binding, and DNA analysis is still unable to detect all suspected mutations (Oo *et al.*, 2013). Dipeptidyl peptidase intravenous (IV) inhibition, in which an inhibitor of these enzymes blocks the hormone from degrading, increases insulin secretion, and lowers blood sugar levels, is one of the most efficient treatments for T2DM. Therefore, glucosidase enzymes play a role in controlling blood glucose levels by delaying the monosaccharide absorption and avoiding a meal's abrupt spike in blood sugar levels (Zabidi *et al.*, 2021). The chemical makeup of chosen ligands, the locations of each receptor's active pocket regions, α -glucosidase, Dipeptidyl peptidase and insulin receptor are displayed in Figure 1.7.

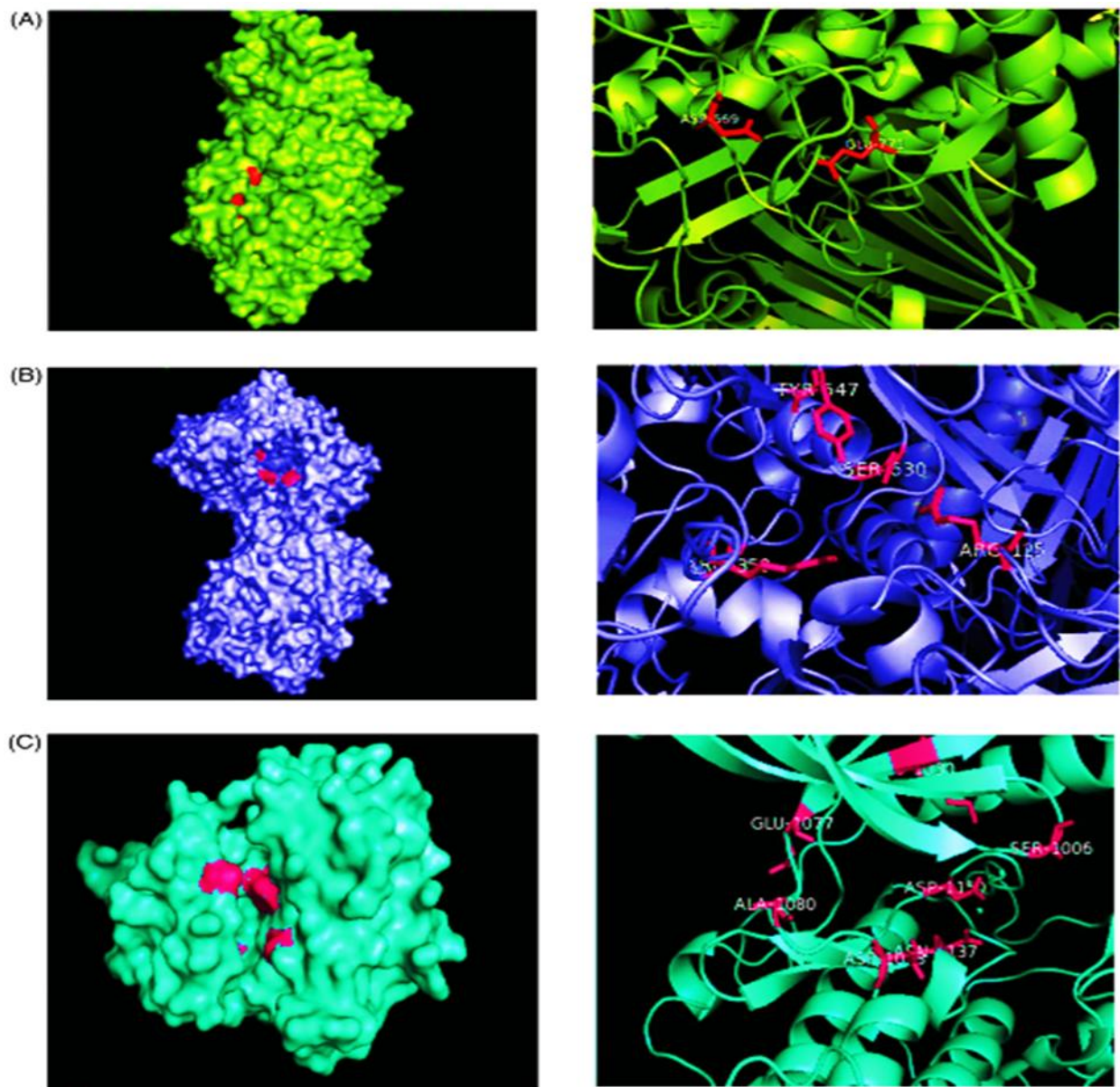


Figure 1.7: Each targeted protein contains an active site. Structure of the α -glucosidase enzyme surface (A), showing the active site of the protein and the active pocket site (red). (B) The structure of the DPP (IV) enzyme surface, showing the active pocket site and the protein active site. (C) The structure of the insulin receptor (green) with the protein's active site and active pocket site. Adopted from Zabidi *et al.*, (2021).

1.5.4 Response of T2DM to COVID-19

During this study period, novel corona virus affected different people in different regions worldwide. Severe inflammatory responses were more likely to be seen in the COVID-19 individuals who had diabetes. Initial studies found increased severity of coronavirus

disease (COVID-19), in patients with diabetes mellitus. Along with raising the risk of developing serious illness, co-morbid conditions such as diabetes, hypertension, obesity, advanced age, cardiac, hepatic, and renal diseases, malignancy, co-infection, immunodeficiency, etc. also raise the risk of dying COVID-19 patients (Hakim *et al.*, 2021). Infected people are predisposed to hyperglycemia, and when it interacts with other risk factors, it may modify inflammatory and immunological responses (Mehta *et al.*, 2020). Diabetes predisposes patients to severe COVID-19, which could have fatal consequences as shown in Figure 1.8. Hyperglycemia conditions support viral proliferation and an increased demand for drugs, length of hospital stay, and death are all expected by poor glycemic control (Lim *et al.*, 2021). COVID-19 patients with T2DM show severe inflammatory response and increase mortality (Malik *et al.*, 2021). Several blood biomarkers such as serum ferritin and HbA1c levels may be routinely used for assessment of progression and severity of COVID-19 in T2DM patients (Malik *et al.*, 2021).

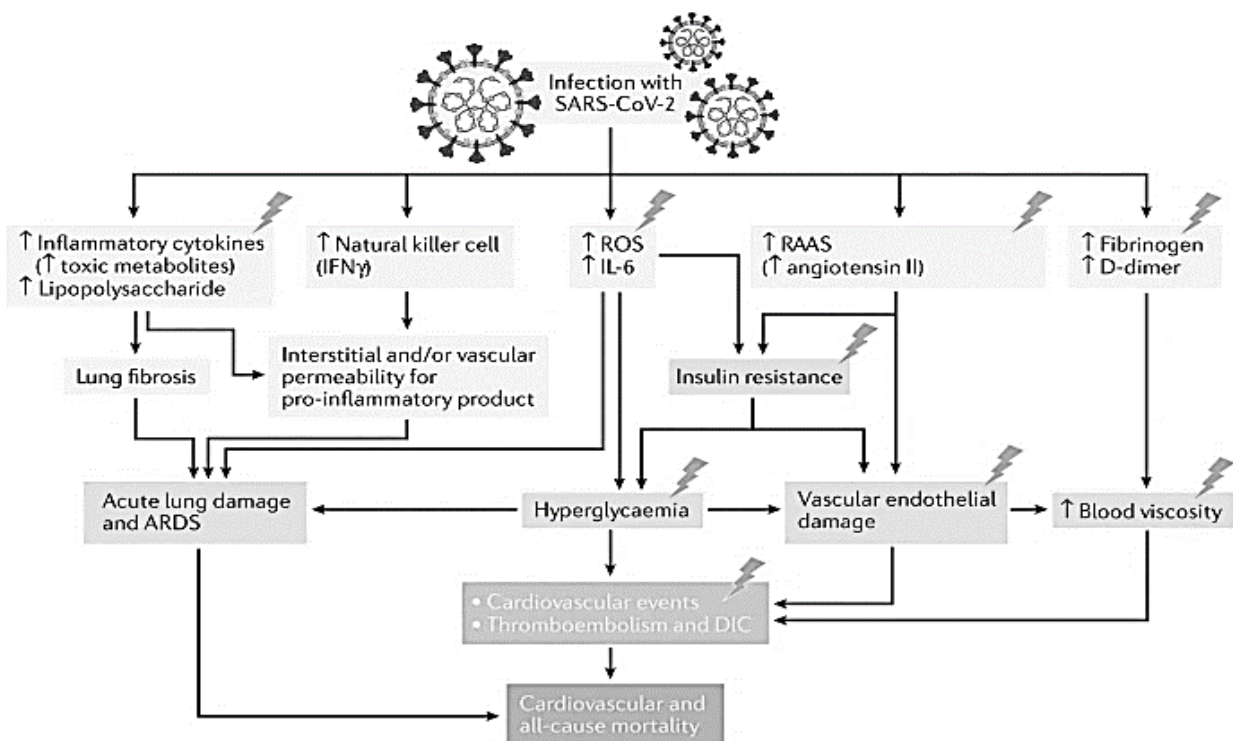


Figure 1.8: Potential pathogenic events in COVID-19 patients with T2DM. Inflammatory cytokines, toxic metabolites, increased production of reactive oxygen species; increased natural killer cell activity together with COVID-19 infection increased vascular permeability contributes to hyperglycemia, endothelial damage and all-cause mortality. Adapted from Lim *et al.*, (2021).

1.6 Literature Review

The most prevalent endocrine illness, diabetes mellitus, causes hyperglycemia due to the inadequate synthesis or action of insulin. In a variety of tissues, including the heart, skeletal muscle, liver and adipose tissue, insulin stimulates the utilization of metabolic substrates under physiological conditions. Normal glucose tolerance is maintained when there is IR or excessive insulin levels due to a number of physiological changes. When there is IR, the pancreas tries to make up for it by secreting sufficient insulin, which leads to hyperinsulinemia (Reaven, 2012). IR is characterized by insulin exerting a biological effect that is less than anticipated (Petersen & Shulman, 2018). By using the GLUT-4 receptors, skeletal muscle and adipocytes respectively contribute for 60–70% and 10%, respectively of the glucose uptake induced by insulin. Furthermore, 30% of the glucose released by insulin is metabolized by the liver, and IR impairs glucose output and fatty acid metabolism, which causes the liver to secrete more triglycerides and VLDL (Czech, 2017). The endothelial balance is maintained by vasodilators like prostaglandin or NO and vasoconstrictors like (e.g., Ang II or ET-1) (Guptha *et al.*, 2014). Adipose tissue is one of the most significant tissues contributing to obesity due to its primary functions of lipogenesis (the storage of fat) and lipolysis (the mobilization of the stored fat) (Janus *et al.*, 2016). FFA concentrations are usually associated with overeating and obesity (Lee *et al.*, 2018).

Vascular homeostasis is maintained by the endothelium, a multipurpose paracrine, autocrine, and endocrine organ. The endothelial balance is maintained by vasodilators like prostaglandin or NO and vasoconstrictors like (e.g., Ang II or ET-1) (Tallapragada *et al.*, 2015). NO also has a protective role for the endothelium by lowering the expression of cell adhesion molecules, increasing platelet aggregation, generating pro-inflammatory cytokines, and limiting the growth of vascular smooth muscle cells (Muniyappa *et al.*, 2020). Selective impairment of PI3K-dependent signaling pathways and decreased vascular and metabolic effects of insulin are crucial aspects of IR at the cellular level, which can lead to hyperglycemia, dyslipidemia and inflammation (Tallapragada *et al.*, 2015). The adhesion molecules of endothelial cells encourage interaction with monocytes, which mature into macrophages and consume lipoproteins to form foam cells that secrete IL-6 and TNF (Lair *et al.*, 2020). Immune cells are activated by TNF- α and IL-6 to form atherosclerotic plaque, which impairs insulin signaling (Czech, 2017). Impaired PI3K

signaling results in diminished endothelial NO synthase activity, pronounced FFA-evoked oxidative stress, and decreased NO bioavailability (Chen *et al.*, 2019). Therefore, relative vasoconstriction and IR are caused by a specific impairment of PI3K signaling (Krishnamoorthy *et al.*, 2020). The phosphorylation of IRS tyrosine activates second messenger phosphotydylinositol-3,4,5-triphosphate (PIP3) (Sakurai *et al.*, 2021). PIP3 aids in the movement of inactive Akt kinase to the cell membrane, where phosphoinoside-dependent kinase-1 turns it on (PDK-1) (Miao *et al.*, 2010). Akt affects angiogenesis, NO generation, and glucose metabolism in addition to cell survival, growth, and proliferation (Manna & Jain, 2015). However, glucotoxicity and lipotoxicity generate inflammation that contribute vascular damage and lead to endothelial dysfunction (Maude *et al.*, 2021). Akt activation in endothelial cells can lead to a decrease in angiogenesis, a reduction in reendothelialization, and unfavorable tumor vasculature growth and survival (Kaur *et al.*, 2018). Chronic inflammation is frequently present in association with obesity and IR, and is primarily induced by the metabolic tissue stress brought on by weight increase and abnormal adipose tissue (Bremer & Jialal, 2013). Hyperglycemia and IR promote increased synthesis of glycosylation end products (AGEs), pro-inflammatory cytokines, oxidative stress, and other inflammatory mediators in addition to enhancing the production of adhesion molecules that control tissue inflammation. Diabetes patients who experience this inflammatory process have an increased risk of infection and poor outcomes (Bohn *et al.*, 2020). Diabetes is a chronic inflammatory condition characterized by multiple metabolic and vascular abnormalities that can affect our response to pathogens. The development of systemic inflammation is the outcome of several physiological changes brought on by obesity (Henning, 2021). There are several hypothesized mechanisms by which virally induced inflammation heightens IR (Zheng *et al.*, 2020). The liver and skeletal muscles, which are the two main insulin-responsive organs in charge of the majority of insulin-mediated glucose uptake, can be negatively impacted by this significant burden of inflammatory cells. When a cytokine storm is present, individuals with viral infection such as severe COVID-19 exhibit muscle weakness and elevated liver enzyme activity, which may indicate multiple organ failure (Fan *et al.*, 2020). Other inflammatory markers such as D-dimer, ferritin, and CRP are elevated in COVID-19 individuals (Grobler *et al.*, 2020; Deng *et al.*, 2021) which may increase the risk of microvascular and macrovascular problems resulting when persons with DM have low-grade vascular inflammation (Lim *et al.*, 2021). A rising role for HDL in regulating immune function has

been revealed by research. In particular, T cells are the primary target of viral action (Paliogiannis *et al.*, 2020). Through the respiratory mucosa, virus particles enter the body. From there, they spread to other cells, unleash a cytokine storm, and set off a chain reaction of immune responses (Sanyaolu *et al.*, 2020). Serological testing can be used to detect antibodies immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA), which are specific for SARS-CoV-2 antigens (Cao, 2020). The current study included a thorough analysis of the connection between illness severity and clinical and biochemical signs. Compared to patients with mild to moderate illness, most critically ill patients were older and had more comorbid conditions. In order to lower mortality and increase recovery rates, it is vital to identify severely ill patients even earlier.

Over the past several years, numerous experts have conducted various investigations to determine the genes that cause T2DM (O'Beirne *et al.*, 2018; Ross *et al.*, 2007; Kashyap *et al.*, 2005). Insulin was the first peptide hormone to be analyzed in receptor binding studies. Most of the binding data may be explained by molecular models of ligand binding to receptors, which have recently been proposed. The exact regions of the receptor that directly contact hormones are not yet defined and are still under investigation (Copps & White, 2012). The two general experimental approaches are being employed to define ligand-receptor contact regions, affinity labeling and mutagenesis (Neff *et al.*, 2020). Mutations of INSR impair insulin responses by reducing the number of INSR on the surface of target cells, or by reducing the receptor's ability to bind insulin (Chadt & Al-Hasani, 2020). The associations of SNPs in insulin secretion and/or T2DM had been examined in many studies (Steinthorsdottir *et al.*, 2007; Kadowaki *et al.*, 1990 Andersen *et al.*, 1992). It should be mentioned that there are diverse association outcomes in the same population as well as distinct association results in different ethnic groups (Wu *et al.*, 2008). INSR is a big warehouse of diseases, e.g. DM which is one of the dangerous one for human beings. Any change or mutation in INSR may change disease pathogenesis. SNPs consist of a single change in the DNA code. Human DNA variations can impact how individuals respond to drugs, chemicals, vaccinations, viruses, and other agents as well as how they develop diseases. Despite not being in protein-coding regions, SNPs can nevertheless affect gene splicing, transcription factor binding, or the sequence of non-coding RNA. The detrimental non-synonymous (nsSNPs) in the INSR gene were examined using a variety of computational methods (D'Alessandris *et al.*, 2004). PROVEAN was

used to start the analysis of the INSR, and then PolyPhen and I-Mutant servers were used to look into the impacts of 57 nsSNPs that were retrieved from the SNP database (dbSNP). The INSR protein structure and function were shown to be adversely affected by a total of 18 SNPs, which were then further examined. According to the computational investigation, 13 of these nsSNP mutations reduced protein stability and may have resulted in functional loss (Longo *et al.*, 2002). Two nsSNPs such as I448T and W1220L (positions rs1051691 and rs52800171, respectively) were predicted as "Highly Destabilizing" (Mahmud *et al.*, 2016). Their inclusion in the INSR raises the risk of diseases caused by the INSR and altered transcriptional and cell cycle control. Human populations vary, therefore, an SNP allele that is prevalent in one region or ethnic group might be substantially rarer in another.

1.7 Purpose of the study`

The study presented herein attempts to better understand the mechanisms by which IR and some biomarkers contribute to metabolic disease progression. It is specifically important in identifying and assessing diabetes risk in the local population. Therefore, the present study was done with the following purposes.

- i. To measure insulin sensitivity, lipid status, obesity in different age groups of Bangladeshi population
- ii. To correlate insulin sensitivity with obese, non-obese and dyslipidemia subjects of different age groups
- iii. To find out blood biomarkers for severity assessment of COVID-19 in diabetic subjects
- iv. To determine the deleterious non-synonymous single nucleotide polymorphisms (nsSNPs) and their impact on the structural integrity of the INSR protein available in the database
- v. To determine nsSNPs in the INSR of Bangladeshi diabetic subjects and compare them with those in the INSR available in the database
- vi. Assessment of the impact of nsSNPs on the three-dimensional structural models of INSR in Bangladeshi diabetic subjects

CHAPTER TWO

Relationship among Obesity, Blood Lipids and Insulin Resistance in Bangladeshi Adults

Summary

Insulin resistance (IR) and abnormal lipid profiles are cardiovascular disease risk factors for obese people. The current study was conducted on a total of 1500 Bangladeshi people at the time of their general health checkup in the hospital to clarify the association between changes in insulin resistance, body weight, and lipid profile. After other endocrine conditions were ruled out, the remaining 772 people were classified as having $IR \geq 2$ and $IR < 2$ estimated insulin resistance based on the homeostatic model evaluation (HOMA-IR) score. Participants free of endocrine disorders were further grouped according to age, gender, and obesity. Statistics were used to assess and link the anthropometric and biochemical profiles with the $IR \geq 2$ and $IR < 2$ groups as well as other subject groupings. All anthropometric data showed considerable changes, with a few exceptions. In the $IR \geq 2$ group compared to the $IR < 2$ group, blood levels of low-density lipoprotein (LDL), total cholesterol (TC), triglycerides (TG), and insulin were all considerably higher. It was discovered that dyslipidemia and obesity were frequent IR components. Obesity and dyslipidemia were shown to be common IR components. IR was significantly impacted by TC: LDL and TG: HDL, according to the generalized linear model. Compared to groups I and III, which include those aged 20 to 40, participants in the age range II (41–60 years old) showed considerably higher lipid profiles (61–80 years old). The findings presented herein support the notion that lipoprotein ratios might be the reliable biomarkers to evaluate IR. Obesity and dyslipidemia were found to be common IR components.

2.1 Introduction

Insulin is an anabolic hormone that regulates blood sugar levels by acting on different target tissues, including the liver, skeletal muscle, and adipose tissues (Petersen & Shulman, 2018). Target cells are affected by insulin via activating the particular receptor. When glucose binds to the insulin receptor protein, the receptor's tyrosine kinase activity is increased, leading to the phosphorylation of several target proteins and facilitating the transport of blood glucose through the plasma membrane (Haeusler *et al.*, 2018). One of the main substrates of the insulin receptor is the adaptor protein IRS-1, which acts as a

platform for the signaling complex. (Beg *et al.*, 2017). A pathophysiological condition known as insulin resistance occurs when cells do not respond to insulin as they should and a particular amount of insulin has less of an effect than is expected. It is characterized by high blood glucose levels and increased hepatic synthesis of atherogenic lipids, can make target tissues less sensitive to the effects of insulin. Other theories contend that it is a genetic and molecular issue that results from impaired insulin communication and glucose transport into cells (Martin *et al.*, 2015). Insulin resistance is a defining feature of obesity, the metabolic syndrome, and cardiovascular illnesses and contributes to the pathogenesis of T2DM (Shao *et al.*, 2018). In South Asians, metabolic syndrome is a major factor in the development of type 2 diabetes mellitus (T2DM) and obesity (Ma *et al.*, 2015). Approximately 20-25% of this metabolic syndrome is in metropolitan South Asians. South Asians in cities have evidence of this metabolic syndrome (Bhardwaj *et al.*, 2011). Effectively implementing and strengthening diagnostic standards is crucial for preventing the "epidemic" of obesity and the metabolic syndrome (Awad *et al.*, 2021). The two conditions of dyslipidemia and insulin resistance are closely related. Greater intracellular hydrolysis of triglycerides (TGs) and release of fatty acids into the circulation are induced by insulin resistance at the level of the fat cell-initiating insult. Because of the molecular or environmental causes of insulin resistance in adipose tissues, there is a reduction in the absorption of free fatty acids (FFA) by fat cells or an increase in the release of FFA from fat cells. Lack of or too much fat causes the liver's fatty acid stability to increase, which in turn causes more very low-density lipoprotein (VLDL) released (Trouwborst *et al.*, 2018). There are various subtypes of low-density lipoprotein (LDL) particles exist. There are two types of tiny LDL, and the differences between them include increased endothelial transport, decreased receptor binding, increased oxidation susceptibility, and increased arterial proteoglycan binding (Chait & den Hartigh, 2020). Compared to other populations, South Asians have higher amounts of LDL cholesterol, and the size of LDL particles is often smaller. Smaller LDL particles are more atherogenic than larger ones due to their higher sensitivity to oxidation. South Asians have lower age- and sex-adjusted HDL levels, which may indicate decreased reverse cholesterol transfer (Kawamoto *et al.*, 2011). Contrary to other conventional risk factors, South Asians consistently have greater rates of diabetes mellitus than many other groups. In actuality, by 2025, Indians will have a greater risk of acquiring T2DM (Bilen *et al.*, 2016). South Asians typically have poor muscle mass, high body fat percentage, abdominal obesity, insulin resistance, and

hyperinsulinemia. Particularly, South Asians frequently have abdominal obesity, which is associated with higher overall insulin resistance and impaired endothelial function in this group (Guptha *et al.*, 2014). Hence, South Asian individuals' body mass index (BMI) thresholds are lower, awareness is growing (Yadav *et al.*, 2013). One may believe that screening procedures for children from South Asia should be modified in accordance with worldwide recommendations (Guptha *et al.*, 2014). However, there aren't many research that have been conducted on Bangladeshi participants to find out how insulin resistance is related to blood lipid profiles, obesity, and obesity, or to find any biomarkers for measuring insulin resistance. The current study's objective was to examine the relationship between insulin resistance, blood lipids, and obesity in Bangladeshi adults between the ages of 20 and 80. According to this study, dyslipidemia and obesity being the most typical causes of insulin resistance.

2.2 Materials and Methods

2.2.1 Population Analysis, Grouping, and Sample Gathering

After getting approval from the North East Medical College Hospital Management, to make sure they would be present for the research process, patients between the ages of 20 and 80 were contacted. 1500 individuals who underwent yearly health examinations from July 2013 to June 2014 provided their histories. Excluded from the analysis were subjects with endocrine disorders, substantial kidney or liver disease, heart disease, cerebrovascular disease, and those using medication for diabetes mellitus or dyslipidemia. Ultimately, we were able to choose 772 individuals who had their anthropometric measurements taken and blood samples were collected from these patients for biochemical analysis. Patients were categorized as $IR \geq 2$ and $IR < 2$ estimated insulin resistance based on the homeostatic model evaluation (HOMA-IR) score after other endocrine diseases were ruled out. Age, gender, and obesity were used to further group the endocrine disease-free participants. Statistics were used to examine the biochemical parameters and anthropometric characteristics and link them with the $IR \geq 2$ and $IR < 2$ both groups and other subjects clustering. All anthropometric data showed considerable changes, with a few exceptions. The grouping of the subjects regarded as a component of the study is shown in Figure 2.1. Before being questioned and physically inspected by a professional team, each subject signed a written

permission. The North East Medical College's ethical review committees in Sylhet, Bangladesh, gave its approval to the procedure.

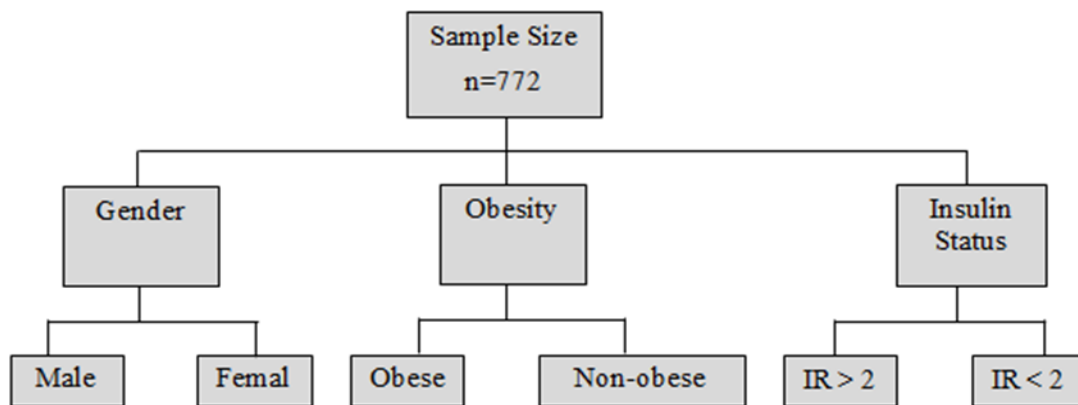


Figure 2.1: Grouping of population of interest for the research

2.2.2 Anthropometric Analyses

An objective survey was used to gather data on demographics, financial situations (education, income), lifestyle (smoking, physical exercise, and food habits), medication usage, personal and family history, and risk factors (hypertension, T2DM, dyslipidemia). Height was calculated to the closest 0.1 cm using a stadiometer set on the wall. Weight was measured to the closest 0.1 kg using calibrated scales. The body weight was adjusted by the height to determine the BMI. The auscultatory method was used to measure blood pressure (Wang *et al.*, 2022). Obesity was classified according to the criteria of WHO based on the BMI value (Bhardwaj *et al.*, 2011).

2.2.2.1 Anthropometric Measurements

Following the prescribed process, height was determined using metric scale and weight was measured using a calibrated weight machine. The participants' Body Mass Indexes (BMI) were computed using the formula below

$$\mathbf{BMI = Weight (kg) / Height (m)^2}$$

2.2.2.2 Measurement of Blood Pressure

Blood pressure of the subject was measured using standard procedure. The calf was placed at the level of the heart and the subject was asked to sit and the reading was taken. Another reading was taken after ten minutes of relaxation. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded according to WHO-HIS (Joseph *et al.*, 2011).

2.2.3 Biochemical Analyses

Standard techniques were used to measure biochemical considerations (including fasting glucose, lipid profile, and insulin). Biochemical assays were performed by a clinical chemistry analyzer (Siemens, Germany) using commercial kits supplied by BIO-RAD laboratories. Radioimmunoassay was used to measure the serum insulin level using a commercial kit (BI-INSULIN IRMA; BIO-RAD, Marnes-la-Coquette, France). The ratios of lipid TC/HDL, LDL/HDL & TG/HDL were calculated as described previously (Laws & Reaven, 1992; Orsi *et al.*, 2021).

2.2.4 Insulin Sensitivity Analyses

Insulin resistance was measured by a homeostasis model assessment (HOMA-IR) index (Fasting plasma glucose [mmol/L] X fasting plasma Insulin [μ U/mL]/22.5), as described previously (Hill *et al.*, 2013). The subjects considering for study based on the optimal cut point for IR for Venezuelan population is 2.00 (Bermdez *et al.*, 2014; Zhang *et al.*, 2021).

2.2.4.1 Homoeostasis Model Assessment (HOMA)

Over the past three decades, Homoeostasis model assessment (HOMA) has been frequently employed as an estimation of insulin sensitivity in cross-sectional, longitudinal and prospective research (Lin *et al.*, 2021). It is based on straight forward fasting measurements of both insulin and glucose and is one of the most commonly used substitute measures of insulin sensitivity (Laws & Reaven, 1992).

$$\text{HOMA} = [\text{fasting insulin } (\mu\text{U/mL})] \times [\text{fasting glucose (mmol/L)}] / 22.5$$

The denominator effectively standardizes insulin and glucose to a normal fasting level [insulin 5 μ U/mL; glucose 4.5 mmol/L]

2.2.5 Body Mass Index (BMI)

Height was calculated to the closest 0.1 cm using a stadiometer set on the wall and body weight was calculated to the closest 0.1 kg using a calibrated scale. BMI was calculated using the following formula-

$$\mathbf{BMI = Weight (kg) / Height (m)^2}$$

Based on the BMI, obesity was categorized according to the guidelines of WHO (Hudda *et al.*, 2019). South Asians have low body mass, high levels of body fat, abdominal obesity, and hyperinsulinemia which are associated with higher overall insulin resistance and impaired endothelial function in this group (Nagao *et al.*, 2013).

2.2.6 Blood Analyses

Biochemical variables (including fasting glucose, insulin and lipid profile) were measured by standard methods.

2.2.6.1 Estimation of Serum Glucose

Serum glucose was measured by the enzyme-dependent colorimetric (GOD-PAP) technique using a commercial kit (Randox Laboratories, UK) (Barham and Trinder, 1972). The tool was calibrated before estimation. Serum and reagents were placed in different cups. These were inserted sequentially into the AUTOLAB analyzer (Analyzer Medical system, Rome, Italy). AUTOLAB analyzer was operated by the manufacturer's instructions and glucose level was calculated using the software provided by the manufacturer.

2.2.6.2 Estimation of Glycosylated Hemoglobin (HbA1c)

Serum HbA1c was calculated using BIO-RAD Kit as described elsewhere (Mayer and Freedman, 1983). The Bio-Rad VARIANT Hemoglobin A1c software used ion-exchange high performance liquid chromatography (HPLC) methods to separate HbA1c automatically and precisely. HbA1c was separated by the D-10 hemoglobin A1c program chromatography using a cation exchange cartridge. Separation was designed to reduce interference from carbamylated hemoglobin, labile A1C, and hemoglobin variations. Elution buffers and wash solution were kept to reach room temperature (15–30°C) before

starting the test. Each chromatogram printout includes a chart that details all of the peaks discovered along with their relative percent and retention times. Samples were diluted with hemolysis reagent and then incubated for a minimum of 30 minutes between 18-28°C. An integrated integrator was embedded into each study's raw data reduction process. The auto-analyzer ran the samples. The calibration response factor for HbA1c was automatically determined after calibrator analysis. Each sample gave a chromatogram and a sample report. The A1c peak was obscured. An exponentially modified Gaussian (EMG) technique was used to compute the A1c peak area, which was then subtracted from the carbamylated and labile A1c peak regions.

2.2.6.3 Estimation of Lipid Profile

2.2.6.3.1 Estimation of Total Cholesterol

AUTOLAB analyzer (Analyzer Medical System, Rome, Italy) was used to quantify total cholesterol. After oxidation and enzymatic hydrolysis, the cholesterol was identified. In a different unit of the analyzer, serum and reagents were collected. The manufacturer's formula and installed software package were used to determine the total cholesterol of the sample.

2.2.6.3.2 Estimation of Triglycerides

Serum triglycerides were measured using commercial kit (Randox Laboratories, UK) in an AUTOLAB analyzer (Analyzer Medical System, Rome, Italy) by the enzyme dependent colorimetric (GPO-PAP) technique as described previously (Trinder, 1969). Triglyceride concentration was computed according to the manufacturer's instructions.

2.2.6.3.3 Estimation of High-Density Lipoprotein (HDL)-Cholesterol

The HDL-Cholesterol levels in blood samples were calculated using the software CHOD-PAP and the testing tools (Randox Laboratories, UK). Chylomicrons, Very Low-Density Lipoproteins (VLDL), and High-Density Lipoprotein cholesterol (HDL) were measured from serum samples using manufacturer's instructions.

2.2.6.3.4 Estimation of Low-Density-Lipoprotein (LDL)-Cholesterol

LDL-C level was calculated using the Friedewald formula (Friedewald *et al.*, 1972) for low-density lipoprotein (LDL)-cholesterol.

2.2.6.4 Estimation of Serum Insulin

Quantitative measurement of serum insulin was carried out by Microparticle Enzyme Immuno Assay (MEIA) in AxSYM System auto analyzer (David *et al.*, 1998), Commercial AxSYM insulin estimated kit was used to estimate serum insulin and AxSYM auto analyzer was operated following the manufacturer's instructions.

2.2.8 Statistical Analyses

Statistical analyses were performed and the results were expressed as a mean \pm standard deviation (SD). P-value <0.05 was considered statistically significant. All variables were checked for normality and Pearson's correlation coefficients were used to identify significant association between anthropometric and biochemical parameters. Correlation co-efficient was performed to examine correlation between various parameters. To identify which set of variables best explained the variation, we examined several combinations of these variables in regression models. Predictors for blood insulin and TG were identified by multiple linear regression analysis. The differences between arithmetic means were assessed using t-test (when two groups were compared) or one-way analyses of variance (when three groups were compared). Complete data analysis was performed using statistical software R (<https://cran.rstudio.com/bin/windows/base/old/3.1.2/>).

2.3 Results

2.3.1 Anthropometric and Biochemical Events Associate with Insulin Resistance

In the first phase of our investigation, 1500 individuals were enrolled. We examined 772 participants for our evaluations after excluding patients with other endocrine disorders (thyroid disorder, pancreatic disease), as they only had the target conditions of obesity and DM. These patients were classified as insulin sensitivity and insulin resistance; male and female; obese and non-obese subjects. The characteristics of the participants were mentioned in Table 2.1.

Table 2.1: Subject characteristics subdivided by insulin status

Variable	Insulin Status			
	IR>2	IR<2	t-value	p-value
Age	52.60±14.44	54.09±15.51	1.29156	0.197
Height	158.74±6.34	159.34±6.53	1.21853	0.223
Weight	62.43±7.11	63.36±7.03	1.73076	0.084
BMI	26.04±2.57	26.22±2.56	0.92032	0.357
SBP	124.62±12.39	123.30±12.04	-1.43	0.0003
DBP	64.84±8.24	64.41±8.09	-0.6999	0
TC	193.80±57.07	174.66±50.31	-4.7572	0
HDL	34.89±10.10	40.16±10.81	6.52477	0
LDL	122.72±46.64	108.93±43.17	-4.0731	0.000053
TG	214.39±136.36	154.77±97.03	-6.9762	0
Non-HDL	158.90±54.73	134.51±48.88	-6.2762	0
TC: HDL	5.90±2.15	4.60±1.69	-9.1611	0
LDL: HDL	3.71±1.51	2.89±1.39	-7.4935	0
TG: HDL	7.14±6.94	4.33±3.58	-7.3821	0
FBS	7.45±2.94	5.21±0.84	-15.893	0
IR	4.49±2.81	1.53±0.28	-23.378	0

The mean ages of insulin resistance groups were 52.60±14.44 and insulin sensitive groups were 54.09±15.51 years. Mean BMI (kg/m²) was 26.04±2.57 and 26.22±2.56 in insulin resistance and insulin sensitive groups respectively. The systolic and diastolic blood pressures were 124.62±12.39 & 123.30±12.04 and 64.84±8.24 & 64.41±8.09 in insulin resistance and insulin sensitive groups respectively. Significant differences in age, BMI, systolic and diastolic blood pressure were present in both groups. On groups of people with insulin resistance (IR_≥2) and insulin sensitivity (IR<2), we assessed the influence of several factors. In comparison to the insulin responsive group, both insulin secretion and sensitivity were considerably decreased in the insulin resistance group. In this study, the HOMA-IR and fasting insulin were used as markers of insulin resistance and risk of upcoming metabolic events. Fasting insulin and the HOMA-IR were utilized in this investigation as indicators of insulin resistance and risk of upcoming metabolic events. Table 2.1 displayed the anthropometric and biochemical characteristics of the insulin

sensitivity and resistance groups. All anthropometric data revealed significant differences. The age difference between insulin sensitivity and insulin resistance groups, however, was not considerably different (54.09 ± 15.51 , 52.60 ± 14.44). The blood pressure in the insulin resistance group was considerably greater than the insulin sensitive group. The level of p-value < 0.05 indicated significant in all cases. Lipid profile of insulin resistance subjects were TC (193.80 ± 57.07), TG (214.39 ± 136.36), LDL (122.72 ± 46.64) & HDL (34.89 ± 10.10) and insulin sensitive subjects were TC (174.66 ± 50.31), TG (154.77 ± 97.03), LDL (108.93 ± 43.17), HDL (40.16 ± 10.81) respectively. $IR \geq 2$ group members had substantially higher total cholesterol, TG, low density lipoprotein (LDL), and fasting serum insulin than $IR < 2$ individuals. We observed substantial changes in LDL, HDL, and TG when evaluating the consequences of insulin resistance and lipid status. Similar investigation, however, only found a substantial impact of TG: HDL in the lipid ratio. Low plasma HDL cholesterol indicates that the quantity and/or size of HDL particles have decreased. Low HDL may have a significant effect on immunity since it performs a number of anti-inflammatory and immune-regulatory actions (Mora, 2013). The TG between the obese and non-obese groups showed a significant difference among the all examined biochemical markers shown in (Table 2.2).

Table 2.2: Subjects' characteristics by obesity category

Variable	Obesity			
	Obese	Non-obese	t-value	p-value
Age	54.71 ± 14.161	49.36 ± 15.634	-4.4801	0
Height	158.56 ± 6.112	159.84 ± 6.958	2.42604	0.0157
Weight	64.98 ± 6.063	57.60 ± 6.597	-14.582	0
BMI	27.34 ± 1.972	23.25 ± 1.099	-36.624	0
SBP	125.07 ± 10.798	122.13 ± 14.963	-2.7038	0.0072
DBP	65.24 ± 7.983	63.43 ± 8.490	-2.7703	0.0059
TC	190.18 ± 55.025	180.60 ± 56.235	-2.1793	0.0298
HDL	37.12 ± 10.711	35.75 ± 10.404	-1.6572	0.0982
LDL	118.78 ± 44.576	116.26 ± 48.916	-0.6731	0.5013
TG	200.03 ± 136.845	179.83 ± 100.921	-2.2738	0.0233
Non-HDL	153.06 ± 53.707	144.85 ± 54.234	-1.9278	0.0545

TC: HDL	5.49±2.085	5.36±2.129	-0.7454	0.4564
LDL: HDL	3.42±1.492	3.45±1.587	0.26479	0.7913
TG: HDL	6.32±6.363	5.85±5.631	-1.0142	0.311
FBS	6.60±2.318	6.88±3.305	1.17681	0.2401
IR	3.46±2.689	3.53±2.687	0.31192	0.7552

2.3.2 Lipid Phenotypes in Insulin Resistance

By improving subject sorting and enabling evaluation based on biochemical and anthropometric factors, cluster analyses remove bias seen in preset variables and cut-off points. Multiple logistic regressions were carried out to examine the relative importance of different variables in affecting blood lipid levels or insulin sensitivity. Regression analyses were carried out for all subjects' lipid parameters and lipid ratio with IR (Table 2.3).

Table 2.3: Analysis of multiple regression of lipid status and lipid ratio with the total number of individuals with insulin resistance

Parameters	Lipid status				Lipid Ratio			
	Estimate	Std. Error	Z value	P value	Estimate	Std. Error	Z value	P value
Intercept	2.33886	1.16841	2	0.045*	0.45116	1.11673	0.4	0.6862
Gender	-0.255	0.17086	-1.49	0.136	-0.254	0.16629	-1.53	0.1266
Age	0.00582	0.00584	1	0.32	0.00412	0.00579	0.71	0.4763
BMI	-0.0491	0.03249	-1.51	0.131	-0.0536	0.03241	-1.65	0.0981
Sys	-0.0051	0.00719	-0.71	0.476	-0.0043	0.00704	-0.61	0.5411
HDL	-0.0486	0.00849	-5.73	1.0e-08***	0.20015	0.14276	1.4	0.1609
LDL	0.00932	0.00204	4.57	5.0e-06***	0.14705	0.15207	0.97	0.3336
TG	0.0044	0.00094	4.7	2.6e-06***	0.10035	0.03554	2.82	0.0048**

*Level of significance <0.05

**Level of significance <0.01

***Level of significance <0.001

We found significant differences on LDL, HDL & TG. However, similar analysis done for the lipid ratio showed only significant effect of TG: HDL. We fitted logistic regression

model of diabetic (FBS>7) and non-diabetic (FBS<7) subjects on TC: HDL, LDL: HDL, TG: HDL. The results revealed significant effect on TC: LDL and TG: HDL shown in Table 2.4.

Table 2.4: Generalized linear model for persons with diabetes (FBS>7) and without diabetes (FBS<7)

Parameter	Estimate	Std. Error	Z value	p-value
TC: LDL	0.533	0.227	2.35	0.01860*
LDL: HDL	0.591	1.090	0.54	0.58746
TG: HDL	1.763	0.480	3.67	0.00024***

*Level of significance <0.05

***Level of significance <0.001

2.3.3 Combination of Dyslipidemia and Insulin Resistance in Participants of Different Ages

To study three age groups with their metabolic health status, patients were divided into three age categories (20-40, 41-60 and 61-80 years). ANOVA was performed to investigate the mean levels of IR for different age groups. A high p-value (0.564) indicated that there was no significant difference in mean IR levels for different age groups. We also performed ANOVA test for mean level of BMI among three age groups of the total population. As we found significant difference (p-value<0.0001) in BMI of three age groups, Tukey's multiple comparison was reported in Table 2.5. Considerable differences between age group 41-60 years (Group I) to 20-40 years (Group II) and 61-80 years (Group III) were observed; however, no significant difference was found between Group II to Group III. Age group related insulin resistance was investigated. The prevalence of insulin resistance significantly varied among the age groups. Changes in age related insulin resistance were shown in Fig. 2.2. Among the three age groups, Group II subjects had significantly higher insulin resistance. Group II was more significantly raised by metabolic factors than others. To correlate the age group related insulin resistance to dyslipidemia, we investigated lipid profiles in all the three age groups.

Table 2.5: Multiple comparisons using Tukey's analysis of the mean BMI with respect to several age groups

Age Group	Difference	Lower	Upper	p-value
(41-60) & (20-40)	0.8687635	0.3348111	1.4027158	0.0004223
(61-80) & (20-40)	1.0100284	0.4190067	1.6010501	0.0001943
(61-80) & (41-60)	0.1412650	-0.3731842	0.6557141	0.7953485

Lipoprotein abnormalities are caused by compensatory hyperinsulinemia and insulin resistance, both of which are primarily brought on by central obesity. Obesity results in increased fasting and postprandial triglyceride-rich lipoproteins, reduced HDL, and increased small dense LDL particles, which together make up the three main components of dyslipidemia. Age-related insulin resistance modifications were reported in (Figure 2.2). Among the three age groups (Group 1: 20-40) years; (Group 2: 41-60) years; (Group 3: 61-80) years; risk factors were comparatively elevated in Group 2 than in the other groups.

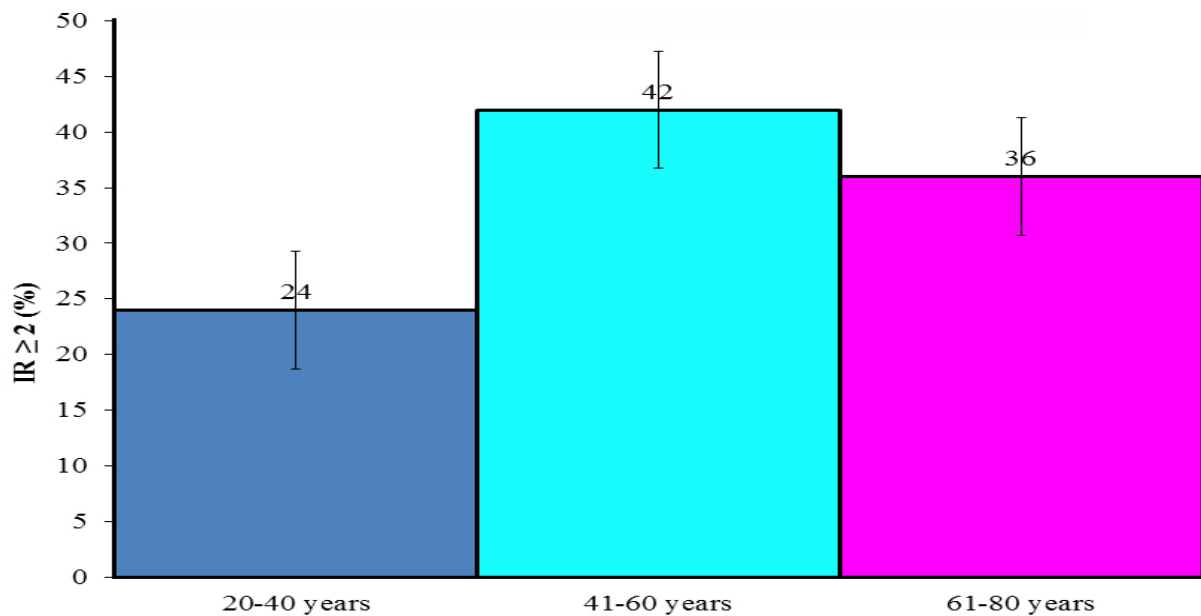


Figure 2.2: Percentage of insulin resistance based on age group

The subjects in Group II displayed the highest levels of insulin resistance among the three age groups. We looked at the lipid profiles in all three age groups to associate the age-related insulin resistance to dyslipidemia. According to the data, group II insulin resistant participants' lipid profiles were significantly greater than those of the other ages (Figure 2.3).

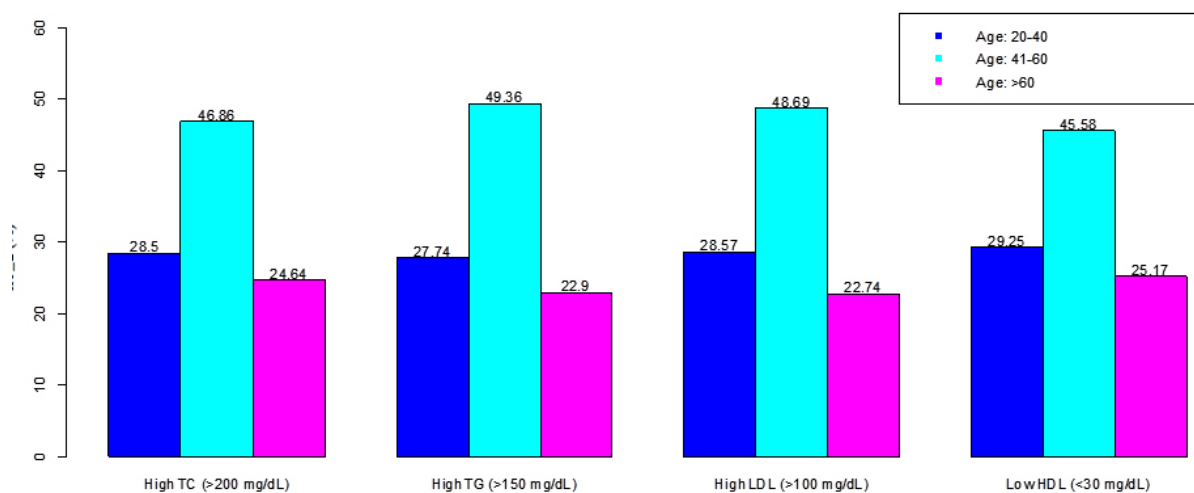


Figure 2.3: Lipid profiles of insulin-resistant individuals of three different age groups

For any level of dyslipidemia most subjects have a higher lipid profile of the middle age group (Figure 2.3). Since other variables like excessive adiposity and dyslipidemia have an impact on and are linked to insulin sensitivity. In this work, we used regular biochemical and anthropometric measurements to construct an estimate of insulin sensitivity. We found that, in both the male and female groups, obese people had considerably lower HDL-C and higher TG levels than non-obese people in terms of their lipid profiles. In a spearman correlation study, we discovered a positive association between weight and BMI and blood sugar, insulin, and HOMA.

Significant positive correlations were observed for insulin and HOMA with glucose and TGs and significant negative correlations were observed between HOMA and glucose as well as HOMA and insulin were not unexpected because HOMA values were derived from insulin and glucose concentration.

2.4 Discussion

This study shows that subjects with insulin resistance are associated with a change in lipid phenotype expression. Recent research identified that a genetic component influences the severity of insulin resistance besides the traditional environmental influences such as BMI (Zhu *et al.*, 2020). In the present study, we demonstrate that subjects with hyper TG were more insulin resistant. This could be related to the increased BMI frequently reported in hyper TG subjects (Fan *et al.*, 2019). Studies reviewed that subjects with dyslipidemia are more insulin resistant, even after-correlation with age, sex and BMI. It has been verified

that cut-off values of BMI should be lower for South Asians than the current worldwide value. According to concept of genetic origin, insulin resistant is associated with an increase LDL particle number and triglyceride and reduction in HDL cholesterol in South Asian population. Increased BMI indicated subjects with hypertriglyceridemia and after correction for BMI, subjects with hypertriglyceridemia are more insulin resistance compare to the hypercholesterolemia. This finding suggests that several metabolic pathways proceed toward abnormal lipid parameters. Scientists resume that insulin resistance or obesity do not entirely account for the higher lipid ratio and also not for the increased prevalence of dyslipidemia. Insulin resistance is strongly associated with measures of obesity, such as BMI. Indeed, insulin resistance is linked by factors other than obesity, as defined by elevated BMI. Circulating IL-6 concentrations increased with obesity predicted to develop type T2DM (Borg *et al.*, 2021). Further studies should be undertaken to investigate the systematic differences between insulin-resistant individuals with high BMI. Insulin resistance was hypothesized to play a major role in dyslipidemia in T2DM. Studies have shown a link between dyslipidemia is the most prevalent component of metabolic syndrome but exact basis of this link is not clear (Silva *et al.*, 2020). It was reported that elevated triglycerides, and LDL cholesterol, and low levels of HDL cholesterol were present in obese adults (Ding *et al.*, 2016). However, in some studies, similar lipid profiles have been reported in obese and non-obese adults with T2DM, in obese normoglycemic adults, and in non-obese adults with impaired glucose tolerance (Pereira *et al.*, 2015). The present study shows that subjects with obese are more insulin resistant compared with controls. Obesity is a cause of insulin resistance, but is also strongly associated with hypertension, dyslipidemia and glucose intolerance. The people with the highest ratio of triglycerides to HDL had 16 times risk of heart attack those with the lowest ratio of triglycerides to HDL (Lee *et al.*, 2016). Since this study also revealed the confirmation of abnormal lipid profile and insulin resistance in the obese adults, which, if not controlled may develop a cardiovascular disease, T2DM and other disorders in later life (Hartogh *et al.*, 2022). Hence it confirms the hypothesis that overweight and obese adults have higher fasting plasma glucose, fasting insulin level and abnormal lipid profile relative to their leaner person. In summary, the routine biochemical analyses performed to date have been unable to provide explanation for the insulin status observed in insulin resistance subjects (Fruman *et al.*, 2017). So, insulin resistance and obesity do not perform unique role for the altered lipid parameters. Many physiological concept and genetic

determinants also reported previously (Smetanina *et al.*, 2021; Pearson *et al.*, 2016). We hope that within the next decade, scientific questions need to be addressed for us to have a more complete understanding of the relationship among obesity, blood lipids and obesity in Bangladeshi adults (Guptha *et al.*, 2014). According to studies, more people who have dyslipidemia are insulin resistant, even after adjusting for age, sex, and BMI. Insulin resistance and obesity were more prevalent in those with higher BMIs. Numerous studies have found that BMI substantially correlates with body fat percentage and is mostly independent of height, enabling meaningful comparisons of groups that are short and tall (Vikram *et al.*, 2008). Body composition and fat free mass are also connected to BMI. Asian populations have higher body fat percentages and higher rates of disease (diabetes, hypertension, dyslipidemia) and mortality with lower BMI (Misra & Shrivastava, 2013).

It has been established that the cut-off values for BMI for South Asians needs to be less than the existing global norm (Misra & Shrivastava, 2013). According to the genetic origin theory, in the South Asian population, insulin resistance is linked to higher triglyceride and LDL particle counts as well as a decrease in HDL cholesterol. After accounting for BMI, participants with hypertriglyceridemia had higher levels of insulin resistance than those with hypercholesterolemia, which was indicated by elevated BMI. This result implies that a number of metabolic pathways lead to aberrant lipid values. South Asians have a higher genetic risk of developing diabetes, although research on metabolic syndrome and insulin resistance has been lacking. Within our analysis, we addressed two restrictions. Anthropometry is not enough to assess health, but the use of statistical techniques enables the accurate information to be filtered out. This study demonstrated a substantial positive link between serum lipoprotein ratios and insulin resistance, suggesting the potential use of these ratios as straight forward, trustworthy, and affordable biomarkers to measure insulin resistance.

CHAPTER THREE

Blood Biochemical Parameters for Assessment of COVID-19 in Diabetic and Non-Diabetic Subjects

Summary

The COVID-19, the ongoing pandemic, is caused by a novel coronavirus named severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2). On March 11, 2020, the World Health Organization (WHO) declared COVID-19 as a pandemic (WHO 2020). In an effort to comprehend the connection between COVID-19 and diabetes mellitus and to assess the most affordable treatment option for the general population, the medical data of all suspected patients from 1 May 2020 to 15 August 2020 in the North East Medical College and Hospital aforementioned were included in the study. The purpose of this study is to identify blood biomarkers for COVID-19 in type 2 diabetics (DM) and non-diabetics (NDM) individuals with the potential to be used to rapidly predict COVID-19 progression and severity. Among 211 hospitalized patients suspected of COVID-19, 98 were confirmed COVID-19 by rRT-PCR. Male people and those with DM were more sensitive to COVID-19, as evidenced by the 58 DM and 40 NDM patients in the COVID-19 positive group having a total of 9 deaths, 7 of whom were male and 6 of whom were DM. Serum ferritin, CRP, D-dimer, ALT, troponin I, and Hb1Ac were among the blood biomarkers that were considerably ($p < 0.05$) higher in COVID-19 individuals. DM COVID-19 patients had considerably higher ferritin and HbA1c levels than NDM patients ($p < 0.05$). According to the results of the current investigation, ferritin, D-dimer, and ALT levels for patients with NDM and DM, respectively, may be routinely utilized as COVID-19 severity and progression biomarkers. Only the poor prognosis of COVID-19 could be detected by CRP and Troponin-I.

3.1 Introduction

The severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV), which is the source of the ongoing pandemic known as COVID-19, is a novel coronavirus (Mallah *et al.*, 2021). The World Health Organization (WHO) declared COVID-19 a pandemic on March 11, 2020 (WHO 2020a). SARS-CoV-2 has been identified as a -coronavirus that, in accordance with research by (Kim *et al.*, 2020, Zhou *et al.*, 2020a, Zhou *et al.*, 2020b).

Severe Acute Respiratory Syndrome (SARS) in 2002–2003 (Xu *et al.*, 2020) and Middle East Respiratory Syndrome (MERS) in 2012 (Rabi *et al.*, 2020) were both brought on by MERS-CoV (Jiang *et al.*, 2020a; Jiang *et al.*, 2020b), respectively. In addition to raising the risk of developing a serious illness, comorbidities including diabetes, hypertension, obesity, older age (more than 60 years), malignancy, co-infection, immunodeficiency, etc. also raise the risk of dying (Ramasamy & Subbian, 2021). When a person meets a surface of an object that is contaminated with SARS-CoV-2 and then touches their nose, mouth, or eyes, the virus can spread (WHO 2020b). Patients with COVID-19 are categorized as (i) mild, (ii) intermediate, (iii) severe, and (iv) critical according on these clinical criteria (Jin *et al.*, 2020). Patients who are seriously ill or in need of acute care must be admitted to intensive care unit (ICU). Comorbidities of patients with COVID-19, such as inflammation, viral myocarditis, myocardial injury and damage, cytopathic effects on kidney tissues, and acute respiratory distress syndrome (ARDS) may be the underlying mechanisms of comorbidity (Lim *et al.*, 2021) shown in Figure 3.1. The gold standard for certifying COVID-19 patients that have been suspected is real-time reverse-transcription polymerase chain reaction (rRT-PCR) (Dhama *et al.*, 2020). However, the rRT-PCR cannot be regularly utilized for the evaluation of the progression and severity, for treatment monitoring, or for the detection of complications related to inherent comorbidities in COVID-19 patients. Because of the cost of the test, it is challenging to every potential patient with this molecular test in time in developing nations like Bangladesh. Serological tests can establish the presence of antibodies known as immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA), which are specific for SARS-CoV-2 antigens. Serological testing is necessary to determine prior SARS-CoV-2 exposure, the prevalence of infection in a region, suspected patients with mild to moderate illness, potential convalescent plasma donors, and the efficiency of plasma treatment (Bhardwaj *et al.*, 2011; Carter *et al.*, 2020; Casadevall & Pirofski, 2020). In order to adopt the proper supportive care and intervention and consequently reduce morbidity and mortality, early assumption and identification of risk factors for severe or critical patients may be highly beneficial. Due to the numerous restrictions mentioned above, especially in developing countries, all suspected individuals cannot access the facility for rRT-PCR testing for confirmation of SARS-CoV-2 exposure. Therefore, blood biochemical profiles during an emergency may be presumptive and beyond initial diagnosis for assessing the severity of the disease, identifying comorbidities, choosing the most appropriate

therapeutic alternatives, and evaluating the effectiveness of the treatments. The link between blood biochemical indicators and assumption, progression, severity evaluation, and therapy monitoring in COVID-19 patients hasn't been well studied, nevertheless (Bohn *et al.*, 2020). In the current study, a number of blood biochemical parameters were examined in order to discover biomarkers for the early diagnosis of type 2 diabetes mellitus (DM) and non-diabetes mellitus (NDM) in COVID-19 patients and to predict comorbidities (Zu *et al.*, 2020).

COVID-19 Patients with Diabetes in poor outcome

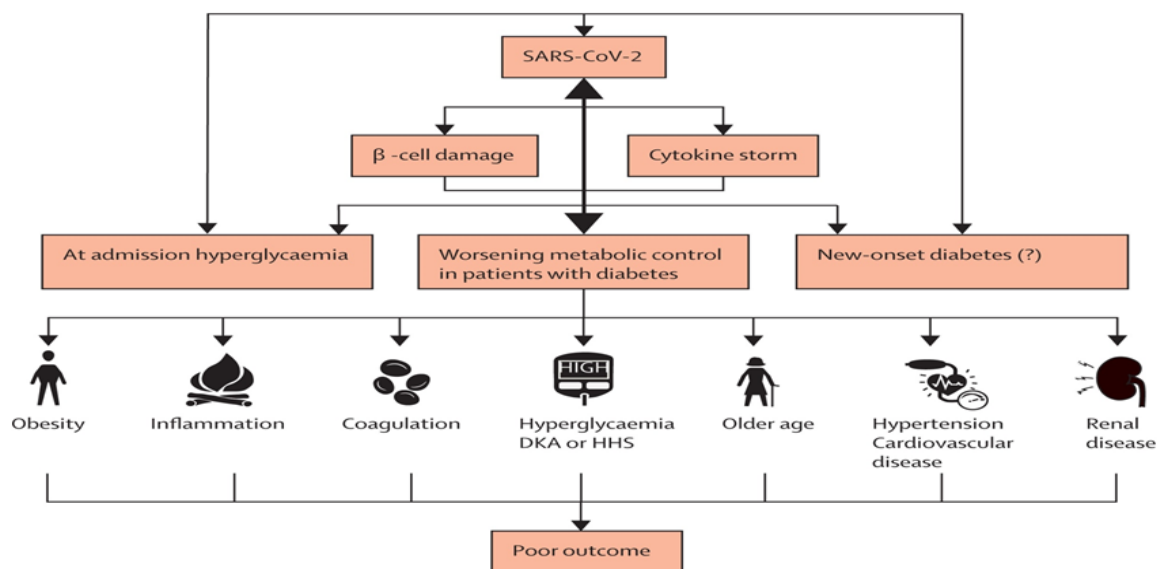


Figure 3.1: Reasons of diabetes with COVID-19 patients. Adapted from Apicella, (2020).

This cross-sectional study was done on suspicious COVID-19 patients who were enrolled between May 1 and August 15, 2020 at the North East Medical College Hospital COVID center, one of the specialists COVID Hospitals in the north-eastern region of Bangladesh. The following exclusion criteria used when patients were admitted to the COVID center: (1) missing data on clinical findings; (2) missing data on laboratory characteristics; (3) secondary diabetes; and (4) type 1 diabetes. In the research, suspected COVID-19 individuals with first clinical symptoms such as a sore throat with breathing difficulty, cough, fever, headache, muscle aches, etc. The population was split into groups for the research, as indicated in Figure 3.2. All of the chosen patients were split into two groups

DM and NDM and had their SARS-CoV-2 infection levels determined by rRT-PCR. All test results, whether positive or negative for COVID-19, were promptly submitted to the national database for COVID-19 patients in Bangladesh in accordance with national criteria established by the government. From the computerized medical records of all the suspected patients, demographic information, medical and exposure history, signs and symptoms, laboratory results, and chest X-rays were retrieved. The accuracy of many bloods biochemical parameters was analyzed in all circumstances using the area under the ROC curve (AUC). The North East Medical College Ethical Committee gave its approval to both the study's protocols and findings.

3.2 Materials and Methods

This cross-sectional study was performed on suspected COVID-19 patients who were enrolled at the North East Medical College Hospital COVID center, one of the specialized COVID Hospitals, in the north-eastern region of Bangladesh, between May 1 and August 15, 2020.

3.2.1 Considerations for Choosing Diabetic Patients

The diabetic patients were chosen based on the following criteria: (1) patients with DM as a high-risk group; (2) records that could provide information on the condition of the diabetes; and (3) data that could provide information on the age and sex of patients who had complications from their diabetes.

3.2.2 Exclusion Criteria

Evidence of hepatic failure, renal dysfunction, or any other type of systemic problem, autoimmune disorder, pregnant mothers, recent cardiovascular disease history, and cancer

3.2.3 Questionnaire

The patient's appointment was planned before the research got underway. Following blood collection, baseline characteristics such as anthropometry and clinical exams were completed. All research participants' information- including age, gender, medical history, academic background, medication information and history of other chronic illnesses, were collected by questionnaire.

3.2.4 Collecting and Storing of Blood Samples

Nasopharyngeal swab samples collected from suspected COVID-19 patients were used to detect the SARS-CoV-2 by rRT-PCR. Blood samples were collected from each participant for analysis of serum electrolytes, lipid profiles, blood sugar, HbA1c, Troponin-I, alanine aminotransferase (ALT), creatinine, C-reactive protein (CRP), ferritin, and D-dimer by using an automated biochemistry analyzer (Siemens, USA).

3.2.5 Anthropometric Measurements

Height, weight and body mass index (BMI) of the subjects were measured as described in chapter two, section 2.2.2.1.

3.2.6 Measurement of Blood Pressure

Blood pressure of the individuals was measured as described in chapter two, section 2.2.2.2.

3.2.7 Biochemical Analysis

Samples from nasopharyngeal swabs taken from suspected COVID-19 patients were used to detect the SARS-CoV-2 by using rRT-PCR. Automated biochemistry analyzer (SIEMENS, USA) was used to measure suspected individual's serum electrolytes, lipid profiles, blood sugar, HbA1c, Troponin-I, alanine aminotransferase (ALT), creatinine, C-reactive protein (CRP), ferritin, and D-dimer.

3.2.7.1 Estimation of Serum Glucose, HbA1c & Cholesterol

Please see the section 2.2.6.1-3 of chapter two for details.

3.2.7.2 Estimation of Serum Creatinine

Serum creatinine was estimated using commercial kit (SIEMENS Laboratories, USA) in a clinical chemistry analyzer (DIMENSION, USA). Creatinine concentration was computed according to the manufacturer's instructions.

3.2.7.3 Estimation of Serum ALT

DIMENSION (clinical chemistry analyzer, USA) was used to quantify serum ALT by the enzyme dependent colorimetric technique using commercial kit (SIEMENS Laboratories, USA) Alanine aminotransferase activity were measured from serum samples using manufacturer's instructions.

3.2.7.4 Measurement of Troponin I

An immunometric approach was employed with the Troponin I calibrators on the VITROS immunodiagnostic system. Serum Troponin I of individual was measured using VITROS immunodiagnostic system (Ortho Clinical Diagnostics, US) following the manufacturer's instructions.

3.2.7.5 Estimation of Serum Ferritin Level

Serum ferritin was measured by ELISA (enzyme-linked Immunosorbent assay) method using Biogen Germany Gmbh ELISA kit. Analyzer was operated by the manufacturer's instructions and serum ferritin was calculated using the software provided by the manufacturer.

3.2.7.6 Quantitative determination of serum CRP by nephelometer

CRP test was measured by using commercial kit (SIEMENS, USA) employs in a clinical chemistry analyzer (DIMENSION Laboratories, USA). The instrument automatically calculated the results in mg/L using calculation scheme illustrated in DIMENSION Operator's Guide.

3.2.7.7 Estimation of D-dimer Level

D-dimer was measured by Immunofluorescence Assay (DIMENSION Laboratories, USA) using D-dimer Fast Test Kit: Getein 1100. The auto-analyzer count down the reaction time and the result printed automatically.

3.3 Statistical Analysis

Exploratory data analyses in the form of boxplots and other 2D and 3D plots were performed on all the variables, and then some statistical test procedures were applied to selected variables. However, only the interesting results are presented in the article.

Differences in the levels of blood biochemical parameters between COVID-19 positive and negative patients with DM and NDM were performed using Student's t-test (McDonald, 2008). Principal Component Analysis (PCA) is a powerful dimension reduction technique, which can also provide a way to separate individuals in a lower dimension (Jolliffe, 2002). PCA was conducted on the variable's ferritin, D-dimer, CRP, and Troponin-I and thereafter a scatterplot of first two principal components was constructed to separate in COVID-19 positive and COVID-19 negative patients.

3.4 Results and Discussion

3.4.1 Baseline Data

For this study, a total of 250 suspected COVID-19 patients were taken into account. 211 of them had their availability of laboratory data examined. A favourable prognosis and minor symptoms were common among these individuals. 211 patients underwent rRT-PCR testing for COVID-19 confirmation. 98 of them had confirmed COVID-19 positive results. The most prominent signs and symptoms in the confirmed COVID-19 patients were fever, cough, and tightness in the chest, tiredness, and shortness of breath. We observed higher numbers of COVID-19 males (n = 60, 61.22%) compared to females (n = 38, 38.78%). Males made up 7 (77.78%) of the 9 (9.18%) deaths in this research with confirmed COVID-19, while females made up 2 (22.22%). This outcome is in line with previous studies (Chen *et al.*, 2020). Variation in the genes of sex chromosomes and sex hormones may be the cause of the sex-biased variation in susceptibility to SARS-CoV-2 infection (Simon 2010; Chen *et al.*, 2020). Estrogen boosts more potent humoral and cell-mediated immunity whereas testosterone inhibits innate immunity (Kovats, 2015; Klein and Flanagan, 2016). Once more, there are around 45.02 (n = 95) and 59.18 (n = 58) DM patients in the total, COVID-19 positive, and COVID-19 negative groups. Those of the NDM patients in the comparable groups are 54.98 (n = 116), 40.82 (n = 40), and 67.26 (n = 76), respectively and 32.74 (n = 37) (Figure 3.2).

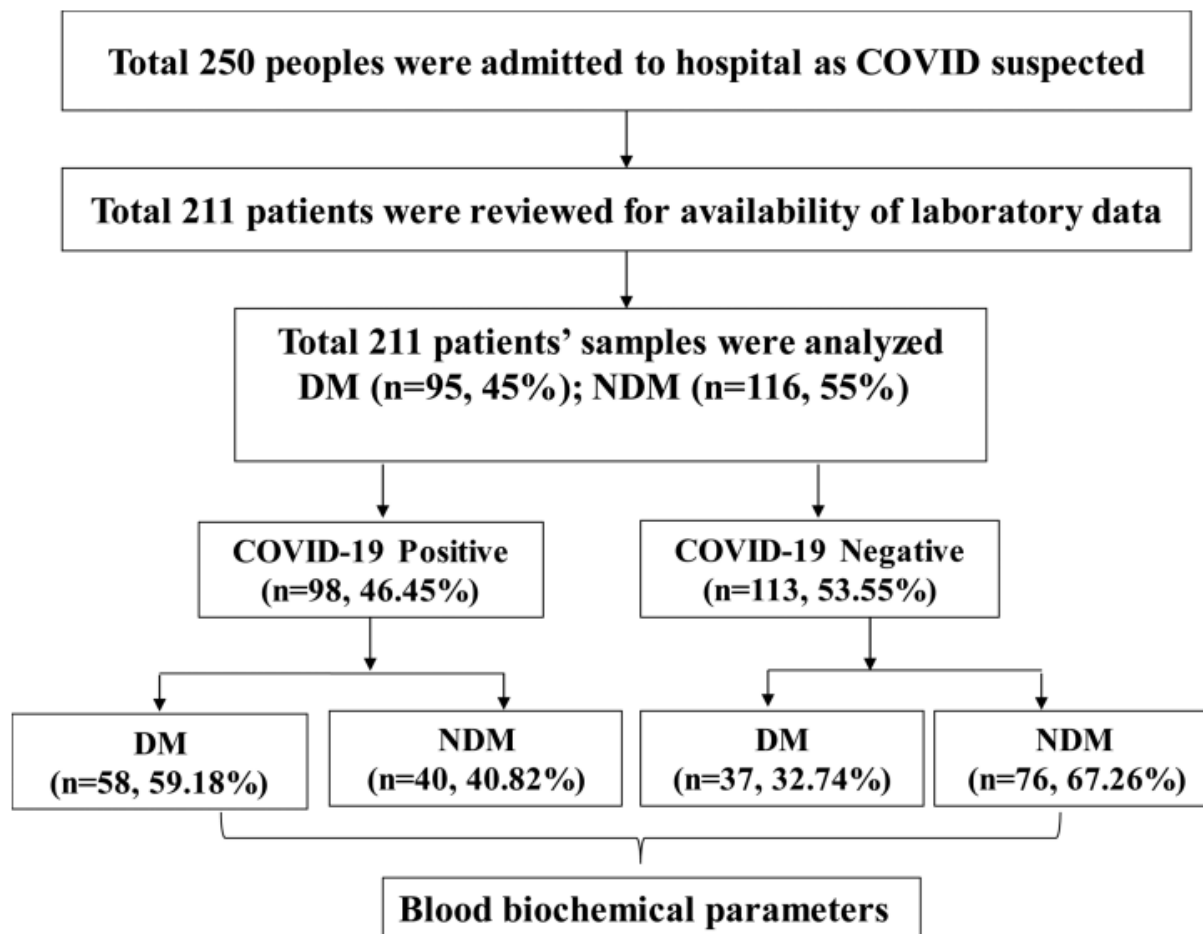


Figure 3.2: Schematic work flow of the study

DM patients made up 6 (66.67%) and NDM patients made up 3 (33.33%) of the 9 deaths with confirmed COVID-19. These findings suggest that SARS-CoV-2 is more contagious among DM patients. Numerous studies have revealed that when DM was present as a separate comorbidity, individuals with COVID-19 had greater rates of morbidity and mortality (Guan *et al.*, 2020; Kumar *et al.*, 2020; Sanyaolu *et al.*, 2020; Wu *et al.*, 2020). The age of the patients ranged from 20 to 80 years with the average age of 54.63 for males and 51.07 for females. Table 3.1 shows that there are no noticeable variations in the mean values of age, height, weight, BMI, systolic blood pressure, and diastolic blood pressure between COVID-19 positive and COVID-19 negative people.

Table 3.1: Baseline parameters of patients with and without COVID-19 (mean values of several factors)

Variable	COVID-19Negative	COVID-19Positive	p-value
Age(yrs), (IQR)	52.28 (27-80)	54.54(26-81)	2.58e-1
Male	74	61	-
Female	40	38	-
Height (cm)	160.39	159.05	1.78e-1
Weight (Kg)	64.83	64.26	6.25e-1
BMI	26.02	26.07	9.14e-1
Sys BP	126.27	125.61	7.36e-1
Dias BP	64.69	64.27	7.56e-1

3.4.2 Blood Biochemical Parameters in COVID-19 Positive and Negative Patients

The biochemical parameters of the COVID-19 positive and negative patients are shown in Table 3.2. In the COVID-19 positive patients, the levels of ALT, HbA1c, D-dimer, CRP, and ferritin were considerably higher ($p < 0.05$) than normal values. Between COVID-19 positive and negative patients Troponin-I was 1.47 and 0.489 respectively. There was a nearly significant difference in the level of Troponin-I ($p = 0.0555$). Other indicators, such as blood sugar, lipid profile, serum electrolytes, and serum creatinine, did not change significantly between the two groups. According to these findings, COVID-19 patients' ALT, serum ferritin, D-dimer, CRP, and Troponin-I levels may be key aspects of disease progression and severity. HbA1c levels were 7.08 and 6.42 in COVID-19 positive and negative individuals, respectively, with a p value of 0.00959 indicating that there would be notable differences between the two groups (Table 3.2). Particularly in cases of severe hyperferritinemia, ferritin plays a significant role in immunological dysregulation by directly suppressing the immune system and promoting inflammation, which causes a cytokine storm (Abbaspour *et al.*, 2014; Son *et al.*, 2019). The severity of the disease is dependent on the presence of cytokine storm syndrome, which is a component of fatal outcomes of COVID-19 (Zhou *et al.*, 2020a). According to the findings of this study,

patients who screened positive for COVID-19 had considerably higher ferritin levels than those who tested negative (Table 3.2).

Table 3.2: COVID-19 negative and COVID-19 positive patients' biochemical findings

Variable	COVID-19 Negative	COVID-19 Positive	p-value
TC (mg/dL)	183.92	176.41	2.79e-01
HDL (mg/dL)	36.56	34.72	1.96e-01
LDL (mg/dL)	118.14	113.42	4.26e-01
TG (mg/dL)	199.69	189.87	5.50e-01
ALT (U/L)	41.88	88.39	7.45e-04
Na ⁺ (mmol/L)	140.27	139.70	5.19e-01
K ⁺ (mmol/L)	4.07	4.13	5.52e-01
Cl ⁻ (mmol/L)	101.57	100.94	3.79e-01
HCO ₃ (mmol/L)	24.05	24.01	9.32e-01
S. creatinine(mg/dL)	1.70	1.70	9.88e-01
FBS (mmol/L)	7.25	7.67	3.23e-01
HbA1C (%)	6.42	7.08	9.59e-03
D-dimer (µg/mL)	1.24	2.76	5.61e-06
Ferritin (ng/mL)	342.13	1031.83	2.86e-23
C-Reactive Protein (mg/L)	59.70	89.27	4.85e02
Troponin I (ng/mL)	0.489	1.47	5.55e-02

Viral and/or bacterial infections result in a rise in ferritin, a marker of infection linked to the immune response and inflammation (Knutson, 2017; Lalueza *et al.*, 2020). The elevated ferritin level seen in 86 (87.76%) COVID-19 patients; males 51 (59.30%) and females 35 (40.70%)-; indicates that serum ferritin may be a potential biomarker for COVID-19 severity and progression of the disease. Some other studies also reported that elevated levels of ferritin might be associated with a composite poor outcome (Huang *et al.*, 2020; Vargas-Vargas and Cortés-Rojo, 2020). D-dimer is generated when plasmin breaks down fibrin to destroy clots created by venous thromboembolism (VTE). It serves as a proximate predictor of thrombin generation and active coagulation during

endovascular thrombotic processes (Linkins and Takach Lapner, 2017). Elevated D-dimer levels in COVID-19 patients may therefore be beneficial in quickly identifying those who have a severe illness, pulmonary problems, and a high risk of VTE (Paliogiannis *et al.*, 2020). In the present study, it was found that the levels of D-dimer were positively correlated with the COVID-19 patients (Table 3.2). About 50% of the suspected COVID-19 patients had D-dimer increases (≥ 0.5 $\mu\text{g/mL}$). One of the possible risk factors for death in adult patients with COVID-19 was hospitalized patients with normal D-dimers ($1\mu\text{g/mL}$) (Zhang *et al.*, 2020). It has been reported that about 50% of the COVID-19 patients with poor prognosis had increased D-dimer levels (Guan *et al.*, 2020; Tang *et al.*, 2020). In the current investigation, individuals with COVID-19 who had increased D-dimer levels were found in 70 (71.43%) of them (41 men, 58.57% women). Only 9 patients passed away, although D-dimer is hypothesized to be associated with a poor outcome (Chen *et al.* 2020; Huang *et al.* 2020; Zhou *et al.* 2020a). The cause of elevated D-dimer levels is multifactorial and its cutoff value has not yet been established in patients with COVID-19. Diverse factors contribute to increased D-dimer levels, and the threshold value for COVID-19 patients has not yet been defined. As a result, the elevated D-dimer level found in a significant proportion of COVID-19 patients (71.43%). The elevated level of D-dimer observed in large numbers of COVID-19 patients (71.43%) indicates that D-dimer test might be useful for COVID-19 assessment and understand the requirement of hospitalization rather than severity of clinical presentation (Thachil *et al.* 2020). As a biomarker for inflammation, cardiovascular disease, and infection, CRP is an acute phase inflammatory protein produced by the liver (Sproston and Ashworth, 2018). It is reported that the elevated CRP level is associated with the severity of COVID-19 (Huang *et al.*, 2020). In this study, COVID-19 positive patients had considerably higher CRP levels than COVID-19 negative patients (Table 3.2). The mean CRP in COVID-19 negative patients is significantly higher than the threshold limit, nevertheless. However, in the current investigation, 91 and 60, respectively, of COVID-19 positive and COVID-19 negative individuals had CRP levels higher than the threshold limit. This finding suggests that the threshold value for COVID-19 patient assessment, monitoring of the disease's progression, and prognostication should be defined. Because several studies utilized various cutoff values, there are discrepancies in the amounts of increased CRP related with the progression and severity of COVID-19 (Ryoo *et al.*, 2019; Huang *et al.*, 2020; Koozi *et al.*, 2020; Liu *et al.*, 2020) reported that the CRP levels increased in 91% of COVID-19

patients on hospitalization and the proportion of patients with increased CRP was significantly higher in severe group than in mild group. Therefore, by setting an optimum cutoff value, the serum CRP level might be used to determine the course and severity of the COVID-19 patients. Elevation of this marker in COVID-19 has been proposed to be an essential biomarker of disease progression and prognosis in myocardial damage (Shah *et al.*, 2020). Results of the current investigation indicated a rather high but marginally insignificant level ($p < 0.0555$) of Troponin-I in patients with COVID-19 than in patients without COVID-19 (Table 3.2). The higher values of the Troponin-I level would be connected with the severity of COVID-19 and the greater trend of the level suggests that it may be a predictor of clinical outcomes. However, only 22 (22.4%) of the COVID-19 patients-14 males (63.64%) and 8 females (36.36%)-had increased Troponin-I levels, mostly in the dead. This finding suggests that the Troponin-I may only be a reliable predictor of COVID-19 severity or poor prognosis. In individuals with severe COVID-19, higher levels of cardiac Troponin-I have been suggested (Lippi *et al.*, 2020). SARS-CoV-2 and SAR-CoV-1 both produce similar clinical symptoms. As the SARS-CoV-1 was found in the liver and induced fatty liver degeneration and central lobular necrosis (Farcas *et al.*, 2005), it is reasonable to believe that SARS-CoV-2 may infect liver. The ALT test was conducted as part of the liver function test. Results revealed that in compared to COVID-19 negative individuals, the level of ALT was considerably higher in COVID-19 patients (Table 3.2). However, ALT levels above the upper limit of the normal range were found in 42 (42.86%) of the COVID-19 patients, indicating that their liver function damage may have been caused by SARS-CoV-2 infection or by symptomatic and supportive antiviral and antipyretic treatments, which may have adverse effects including liver damage (Fan *et al.*, 2020). This result is in agreement with other studies (Cai *et al.*, 2020; Chen *et al.*, 2020; Fan *et al.*, 2020). Hospitalized COVID-19 patients who passed away had ALT levels that were 2 to 15 times higher than the upper limit of the normal range. However, 17 (15.05%) of the COVID-19 negative individuals had ALT levels that were somewhat higher than the upper limit of the normal range. The medications mentioned earlier may have impaired the liver function in these people.

3.4.3 Effects of DM on COVID-19 Positive Patients

When compared to COVID-19 individuals without diabetes, it was shown that patients with DM had substantially higher levels of HbA1c, FBS, and serum ferritin (Table 3.2). Only the DM patients with positive COVID-19 results and those with negative COVID-19 results had their biochemical markers further examined (Table 3.3).

Table 3.3: Biochemical parameters of patients with DM and Non-DM

Variables	Diabetic	Non-Diabetic	p-value
TC (mg/dL)	177.93	182.54	5.10e-01
HDL (mg/dL)	34.33	36.84	6.95e-02
LDL (mg/dL)	117.93	114.37	5.54e-01
TG (mg/dL)	191.87	197.88	7.11e-01
ALT (U/L)	68.73	58.78	4.37e-01
Na ⁺ (mmol/L)	140.74	139.40	1.27e-01
K ⁺ (mmol/L)	4.06	4.14	4.13e-01
Cl ⁻ (mmol/L)	101.80	100.87	1.97e-01
HCO ₃ (mmol/L)	23.48	24.49	4.86e-02
S. creatinine(mg/dL)	1.87	1.56	1.15e-01
FBS (mmol/L)	9.46	5.79	3.72e-16
HbA1C (%)	8.29	5.44	4.05e-32
D-dimer (µg/mL)	2.14	1.78	2.82e-01
Ferritin (ng/mL)	748.80	585.82	3.20e-02
C-Reactive Protein (mg/L)	78.00	69.44	5.45e-01
Troponin I (ng/mL)	1.00	0.88	7.96e-01

The findings demonstrated that DM COVID-19 positive patients had substantially higher serum ferritin, D-dimer, and ALT levels than DM COVID-19 negative patients. This information suggests that DM may have made the immunological dysregulation that caused the cytokine storm worse (Abbaspour *et al.*, 2014), the risk of VTE (Linkins and Lapner, 2017; Paliogiannis *et al.*, 2020) and liver function damage (Cai *et al.*, 2020; Chen *et al.*, 2020; Fan *et al.*, 2020). Serum ferritin, D-dimer, and ALT levels in NDM patients with COVID-19 positive and COVID-19 negative were considerably higher, according to

analysis of the blood parameters in these patients exclusively. Individuals with COVID-19 compared to those with NDM patients who did not have COVID-19 (Table 3.3). FBS levels are often greater in DM patients with uncontrolled disease. In actuality, HbA1c represents the average blood sugar levels over the previous three months, and a higher HbA1c level suggests inadequate glycemic management. In comparison to the NDM COVID-19 group, the mean HbA1c value was considerably higher in the DM COVID-19 group (Table 3.3). However, neither COVID-19 positive nor COVID-19 negative DM patients' levels of HbA1c or FBS showed a noticeable difference (Table 3.3). As a result, (Tables 3.2 and 3.3) findings support the idea that COVID-19 patients with diabetes do not have aggravated diabetes, but rather that the multiple pathophysiological events caused by DM may be related to the progression and severity of COVID-19. This may be a plausible explanation for why COVID-19 patients with DM had a higher possibility of developing serious complications. Despite some differences, all of the patients who died from COVID-19 with DM had levels of ferritin, D-dimer, CRP, and ALT that were many times higher than normal (Table 3.2), indicating that numerous pathophysiological processes were likely involved in their demise. According to a study using meta-analysis, DM in COVID-19 patients twice the morbidity and severity of the condition compared to NDM (Cariou *et al.*, 2020; Pal & Bhadada, 2020; Kumar *et al.*, 2020). Poor results in COVID-19 have been connected to common diabetes demographics and comorbidities such older age, male sex, cardiovascular disease, hypertension, and obesity (Zheng *et al.*, 2020). However, it is still unclear if DM directly affects COVID-19 patients' morbidity and mortality, or whether the cardiovascular and renal comorbidities that are frequently linked to DM are the main variables at play. Results from the current study (Tables 3.2 and 3.3) point to the possibility of routinely using serum ferritin, D-dimer, and ALT levels as biomarkers for COVID-19 progression and severity evaluation in DM patients and NDM patients, respectively. Although there was no discernible difference in CRP and Troponin-I levels, both exhibited a greater trend in COVID-19 positive patients, whether they had diabetes or not (Tables 3.2 and 3.3). This pattern could reflect to the COVID-19 patients' elevated risk of heart failure. Data in (Table 3.2 and 3.3) reveal that these biomarkers ought to be investigated for COVID-19 severity estimation.

3.4.4 Explorative Data Analysis

The boxplots demonstrate that patients with COVID-19 positivity had higher median ferritin levels than COVID-19 negative individuals. But the graph shows no appreciable variation in ferritin levels between DM and NDM people (Figure 3.3).

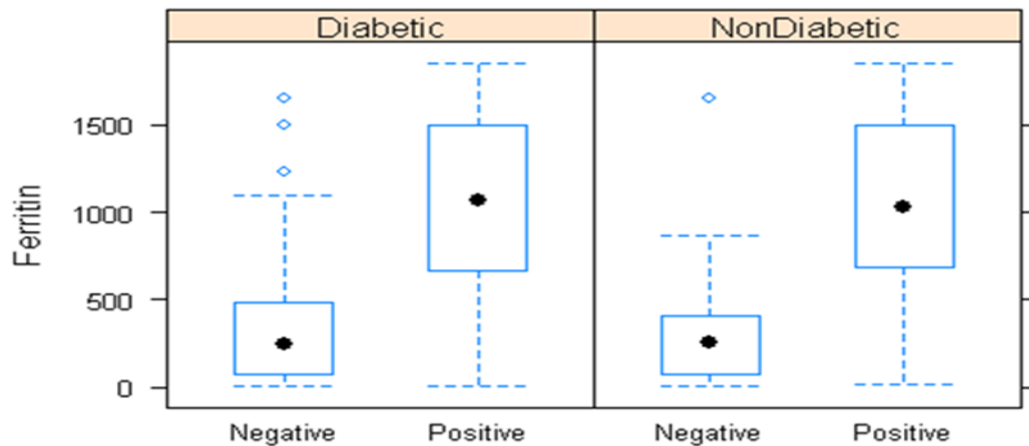


Figure 3.3: Ferritin boxplots in DM and NDM patients with positive and negative COVID-19 findings

We used a simple 3D plot Figure 3.4(a) of these variables and colored the dots according to COVID-19 positive and negative since serum ferritin, D-dimer, and CRP may play major roles in screening COVID-19 positive and negative people. It is noted that none of the points can be fully distinguished by the aforementioned three factors. On the variables ferritin, D-dimer, CRP, and Troponin-I, we conducted principal component analysis (PCA) as an extension of categorization. The PCA analysis also revealed that not all of the variables' points can be entirely separated Figure 3.4(b).

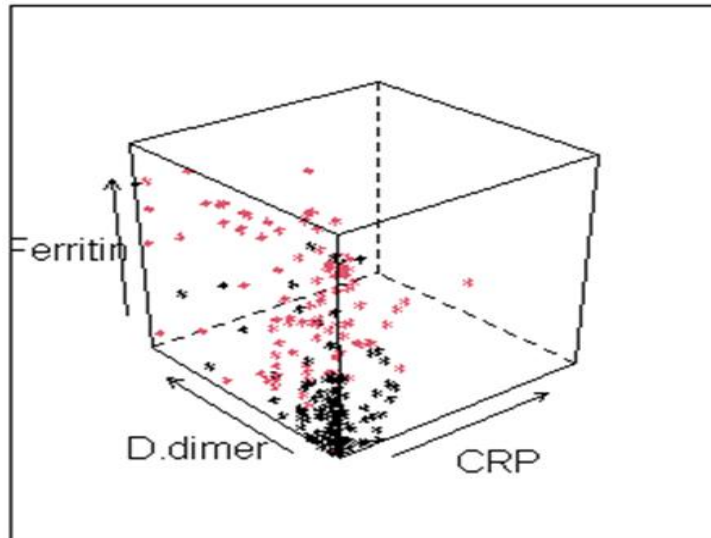


Figure 3.4 (a): 3D plot of Ferritin, D-dimer and CRP as these variables might play role in detecting COVID and non-COVID

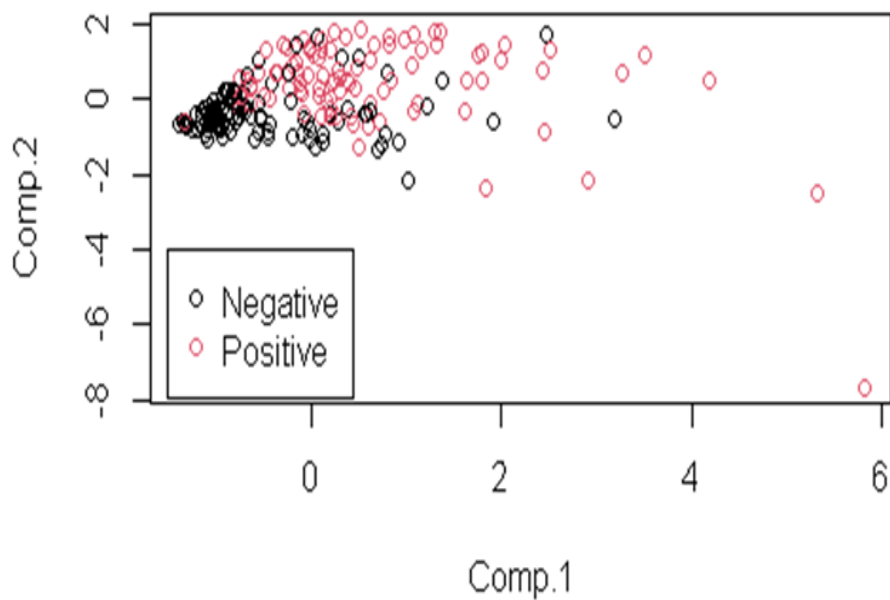


Figure 3.4 (b): Use principal component analysis of variables Ferritin, CRP and d-dimer for partitioning the groups

CHAPTER FOUR

***In Silico* Analysis of Damaging Single-Nucleotide Polymorphisms and Their Structural and Functional Impact on the Insulin Receptor**

Summary

The SNPs in the coding region of a protein may confer structural modification and destabilization for alteration of functions. The SNPs in the INSR are considered as a cause disease namely Donohue syndrome or Leprechaunism, Rabson-Mendenhall syndrome, and type A insulin resistance. Therefore, the deleterious nsSNPs in INSR have been analyzed based on different bioinformatics tools. Initially, the effects of 57 nsSNPs retrieved from database of SNP (dbSNP) were analyzed with PROVEAN followed by PolyPhen and I-Mutant servers. Eighteen mutants predicted to confer damaging effects on the INSR protein structure and function were further analyzed. The computational analysis predicted 13 nsSNPs conferring decreased protein stability loss of function. Two SNPs generating I448T and W1220L were labeled as "Highly Destabilizing" among all the SNPs, suggesting that these polymorphisms should be taken into consideration as a potential target for further research. The single amino acid substitution on the structure of a protein or the interaction between proteins might be significant.

4.1 Introduction

It is estimated that about 270 million adults worldwide have T2DM, and this figure is anticipated to be 416.5 by 2030 (Crafa *et al.*, 2021). In different individuals and groups, a single-gene mutation or SNP does not always result in the same results. A single nucleotide A, T, C, or G in the genome (or other shared sequence) that differs between individuals of a biological species or paired chromosomes is known as a single nucleotide polymorphism. This variation in DNA sequence occurs frequently within a population (e.g., 1%). SNPs can act as a helpful genetic marker for genome-wide association studies. SNPs' repercussions or harmful effects are typically linked to how they affect the structure and function of proteins. The genetic makeup of an individual, a family, or a population as a whole may have an impact on this variance directly or indirectly and may interact with a variety of environmental circumstances (Staiger *et al.*, 2009). In the course of genome-wide association studies (GWAS), more than 70 SNPs comprising more than 40 genomic

regions have been identified, and each one contributes just marginally to a person's chance of acquiring T2DM (Kharroubi & Darwish, 2015). Age, lifestyle changes, decreased physical activity, high-calorie meals, and ethnicity, income, and socioeconomic status are all risk factors for diabetes (Abuhendi *et al.*, 2019). The risk of having diabetes may also be increased by hereditary factors. The exons of the genome are home to roughly 500,000 SNPs, which make up the majority of human genetic variations. These SNPs, particularly nonsynonymous SNPs (nsSNPs), can alter the amino acid residues and increase the functional variety of encoded proteins in the human population (Musambil & Siddiqui, 2019). It is clear that SNPs are not distributed evenly across the chromosomes. Instead of coding sections, SNPs typically exist in non-coding regions (Barreiro *et al.*, 2008). It is currently very difficult to identify the functional SNPs in a disease-related gene using laboratory techniques. Due to recent advancements in the "*in-silico*" technique and procedures, research inquiries can now be conducted without the need for extensive lab work. Because genetic differences are important risk factors associated with diabetes susceptibility, the SNPs in the INSR retrieved from database of SNP (dbSNP) were analyzed. The main goal of this study is to investigate the SNP genetic variations in the human INSR gene and their potential effects on the protein structure and activity using bioinformatics and computational techniques. The SNPs and their effects on INSR, however, have only been the subject of a relatively small number of researches (Thomas *et al.*, 2011).

In the present study, it has been focused to investigate the SNPs in the human INSR gene and their possible effects on structure and functions of INSR using bioinformatics tools. The deleterious nsSNPs in the INSR have been predicted. The impact of the deleterious nsSNPs has been investigated after construction three dimensional (3D) models. These SNPs may have an impact on the structural integrity of human INSR protein and be involved in several genetic diseases.

4.2 Materials and Methods

4.2.1 Datasets

The data of human INSR gene was collected from Online Mendelian Inheritance in Man (OMIM) and Entrez Gene on National Center for Biotechnology Information (NCBI) web sites.

The SNPs information (protein accession number and SNP ID) of the INSR gene was retrieved from the NCBI dbSNP (<http://www.ncbi.nlm.nih.gov/snp/>).

4.2.2 Analysis of Protein Variation Effects

A predictor based on protein sequences calculates the impact of protein sequence variation on function. The impact of single amino acid mutations on protein stability and protein binding effectiveness can be predicted using a variety of web servers. The following programs were utilized in this study: PROVEAN (<http://provean.jcvi.org/index.php>), I-Mutant 3.0 (<http://gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi>), and PolyPhen (<http://genetics.bwh.harvard.edu/pph2/>). Protein sequences from BLAST hits that shared more than 75% of their global sequence identity were grouped together in PROVEAN, and the top clusters were used to create a supporting sequence collection. The final PROVEAN score was calculated using a delta alignment scoring system, which summed the scores of each supporting sequence both within and between clusters. If the final score falls below a predetermined threshold (the default is 2.5), a protein variant is described as "deleterious," and if the score rises over the threshold, it is projected to be "neutral" (Choi *et al.*, 2012). Using precise empirical principles, PolyPhen version 2 forecasts the impact of amino acid substitution on the composition and behavior of proteins. The input choices for PolyPhen include protein sequence, database ID/accession number, amino acid position, and information on amino acid variants (Ramensky *et al.*, 2002). The tool calculates the score difference across variations and estimates the position-specific independent count (PSIC) score for each variant. Support Vector Machine (SVM) algorithm is the foundation of I-Mutant 2.0 and I-Mutant 3.0, which estimate the stability of the protein due to single amino acid mutations. By using the protein sequence or structure, it can forecast changes in protein stability. When prediction is based on protein sequence, its overall accuracy is 77%. As regression estimators, I-Mutant 2.0 and I-Mutant 3.0 forecast the DDG values as well as the stability change's sign. Additionally, I-Mutant 3.0 divides mutations into the following three groups: neutral mutation (0.5 DDG 0.5), large drop (0.5), and significant gain (>0.5) (Abagyan *et al.*, 1994; Schymkowitz *et al.*, 2005).

4.2.3 3D Modeling and Analysis of Protein Structure

The INSR-related proteins were discovered using the EMBL-EBI website tool PDBsum (<http://www.ebi.ac.uk/pdbsum/>). PDBsum offers a quick look at each macromolecular structure that has been deposited in the Protein Data Bank (PDB). To generate a list of the closest matches, it runs a FASTA search against each sequence in the PDB (de Beer *et al.*, 2014). Using characteristics of their local structural surroundings, potential binding interactions, and evolutionary conservation, LS-SNP/PDB (Ryan *et al.*, 2009) annotates all human SNPs that result in an amino acid change in a protein structure in PDB (Deshpande *et al.*, 2005). A highly conserved surface patch or a charged surface patch with a nsSNP suggests potential biological significance. With the aid of these annotations, users can swiftly examine a large number of nsSNPs of interest and give priority to those that are most likely to affect the regular protein activities. To study human nsSNPs into protein homology models, the LS-SNP website is very helpful (Karchin *et al.*, 2005). The mutant models of each of the chosen PDB entries for the relevant amino acid substitutions were created using PYMOL. To modify amino acids, PYMOL enables browsing through a library of rotamers. The natural amino acid was switched out with a different one using a "Mutagenesis Wizard." The "best" rotamer of the new amino acid can be used to replace the native amino acid with the help of the mutation tool. For each model, ".pdb" files were saved.

4.2.4 Structure Validation and Energy Minimization

For assessing the caliber and validation of the improved 3D structural models, the Structural Analysis and Verification Server (SAVES) was built. The PROCHECK, PROVE, and ERRAT software applications are integrated into SAVES to examine the overall quality of the 3D models produced by the PYMOL mutagenesis tool. Using KoBaMIN, a knowledge-based potential refinement for proteins methodology, structure refinement was done (Rodrigues *et al.*, 2002).

4.2.5 Protein Stability Validation for Mutant Structure

To examine and forecast the effects of single-point mutations on protein stability and protein-protein and protein-nucleic acid affinity, a method known as Mutation Cutoff

Scanning Matrix (mCSM) is used. To represent the surroundings of protein residues, the mCSM records distance patterns between atoms (Pires *et al.*, 2014).

4.2.6 Structural Analysis

Chimera at the University of California, San Francisco (UCSF) allowed users to see the anticipated structures. It is a computationally complex tool for molecular model visualization, and it offers the user an interactive interface for studying the models and data associated with the models. It offers a platform for studying sequence alignments, producing homology models, performing molecular docking, visualizing different density models, and superimposing several models for comparison (Pettersen *et al.*, 2004). The effect of the nonsynonymous alteration in terms of steric hindrance due to the changes in the side chains and charge of the amino acid was observed when the mutant and wild type structures were overlaid. The impact of the substituted amino acid's degree of hydrophobicity or hydrophilicity on the interacting intrachain and interchain molecules was then examined. The workflow of this chapter using different bioinformatics tools is shown in Figure 4.1.

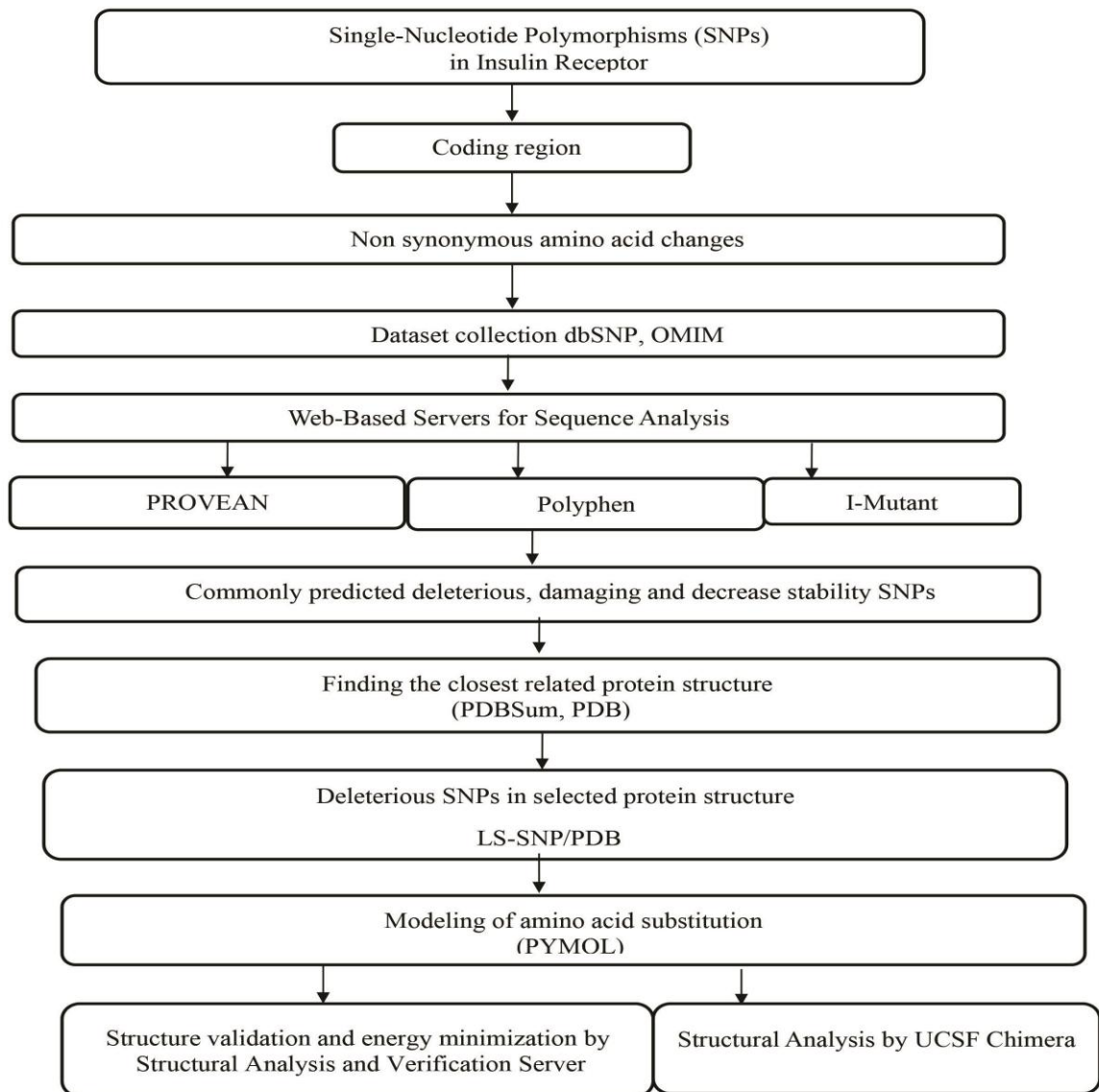


Figure 4.1: A flow chart of bioinformatics tools

4.3 Results and Discussion

4.3.1 SNP Dataset from dbSNP

Both validated and unvalidated polymorphisms can be found in the dbSNP. Despite this flaw, we chose to use the dbSNP because it is the largest SNP database and most of the nsSNPs of INSR have their allelic frequency documented there. Some previously reported SNPs in dbSNP have been found to be invalid in our data search due to improper sequencing and alignment. These incorrect SNPs have either vanished or combined with additional SNPs. The names of some INSR genes have been changed.

The outdated and useless SNPs were eliminated after a thorough cross-examination of the databases. The INSR gene has 4967 SNPs with data available at dbSNP. Only 57 of the 4967 SNPs in the coding area were nsSNPs (Table 4.1). Only the nsSNPs in the coding area were taken into account in our analysis.

Table 4.1: List of nsSNPs that were predicted to have functional significance by PROVEAN

SNP_ID	Mutation	PROVEAN Result	PROVEAN Score
rs1799816	V1012M	Neutral	-2.418
rs52836744	G58R	Deleterious	-6.762
rs121913144	R1027*	-	-
rs121913145	H236R	Deleterious	-5.616
rs121913156	R1201Q	Deleterious	-3.701
rs891087	D261E	Neutral	-0.116
rs2162771	P830L	Neutral	-1.576
rs13306449	Y1361C	Deleterious	-4.157
rs35045353	G811S	Neutral	-2.401
rs1051691	I448T	Deleterious	-3.774
rs1051692	Y171H	Neutral	-1.304
rs2229429	D546E	Neutral	-2.474
rs7508518	A2G	Neutral	0.465
rs52800171	W1220L	Deleterious	-11.648
rs55816055	S353P	Deleterious	-2.761
rs56395521	L1065V	Neutral	-1.133
rs72549237	V362I	Neutral	-0.211
rs76077021	R889W	Deleterious	-4.254
rs76673783	E664G	Deleterious	-5.068
rs78433961	R796S	Neutral	-0.984
rs78827745	M65K	Deleterious	-3.808
rs79312957	R413C	Deleterious	-5.623
rs113527718	S1297G	Deleterious	-2.539
rs138528064	T320M	Neutral	-1.959
rs140762552	T107M	Deleterious	-2.922
rs140852238	E51K	Deleterious	-2.545
rs141484557	G262S	Deleterious	-3.037
rs142391704	A706D	Neutral	0.622
rs142910337	D75G	Neutral	1.672
rs143523271	S748L	Neutral	-0.793
rs143919163	G192D	Neutral	-1.884
rs144029037	V900I	Neutral	-0.698
rs146588336	D946E	Neutral	-0.743
rs147671523	E517G	Deleterious	-4.293

SNP_ID	Mutation	PROVEAN Result	PROVEAN Score
rs148838377	P755S	Neutral	-0.076
rs149536206	H8598*	-	-
rs150114699	L991I	Neutral	-1.71
rs181150880	R410Q	Neutral	-2.011
rs182552223	T858A	Deleterious	-2.612
rs183360558	D893N	Neutral	-1.752
rs185736681	R1053C	Deleterious	-5.933
rs187282966	R889Q	Neutral	-1.492
rs199580495	S1033F	Deleterious	-5.642
rs199599404	M1319I	Neutral	-1.196
rs199659271	C219R	Deleterious	-9.831
rs200059069	K411Q	Neutral	-1.21
rs200110540	V866I	Neutral	-0.134
rs200199169	P271L	Neutral	-2.315
rs200400127	A1340V	Neutral	-1.519
rs200921389	G1048D	Neutral	-1.585
rs201147780	K294R	Neutral	-0.904
rs201466857	T858M	Deleterious	-3.384
rs201506342	P1312T	Deleterious	-3.008
rs201978448	A537V	Deleterious	-3.252
rs201979105	S1221A	Deleterious	-2.698
rs202160383	R1128H	Neutral	-2.063

4.3.2 Effects of nsSNPs on INSR Predicted by Bioinformatics Tools

While other tools function similarly with the structure, the PROVEAN algorithm primarily uses the primary sequence for prediction. In contrast to other tools, PROVEAN has the advantage of being able to forecast a large number of replacements without the need for structures. Based on sequence homology, PROVEAN forecasts how the variant will affect the biological function of the protein. PROVEAN scores are categorized as "neutral" over a given threshold (here 2.5) and "deleterious" below it. To determine the PROVEAN score, a.txt file containing the "db SNP rsIDs" of each of the 57 nsSNPs was uploaded to the "dbSNP rsIDs" page. 24 of the 57 nsSNPs were predicted by PROVEAN to be harmful, and 33 to be neutral (Table 4.1). W1220L and C219R, two of the 24 harmful nsSNP mutations, had PROVEAN scores of -11.648 and -9.831, respectively, and were projected to be very detrimental.

Through the use of BLAST, PolyPhen locates homologs of the input sequences and produces PSIC scores for each variation, estimating the difference between the variant

scores; the difference of 0.339 is harmful. The sequences are subjected to some empirical guidelines, and the accuracy is about 82% with an 8% probability of a false-positive prediction. Each of the 57 nsSNPs' matching amino acid changes and the protein accession number of INSR (P06213) were submitted separately. The results from the PolyPhen server are compiled in (Table 4.2).

Table 4.2: Potential effect of amino acid substitution for nsSNPs in human INSR gene predicted by the PolyPhen algorithm

SNP_ID	Mutation	Polyphen Result	Score	Sensitivity	Specificity
rs1799816	V1012M	PROBABLY DAMAGING	0.992	0.7	0.97
rs52836744	G58R	PROBABLY DAMAGING	1	0	1
rs121913144	R1027*	-	-	-	-
rs121913145	H236R	PROBABLY DAMAGING	1	0	1
rs121913156	R1201Q	PROBABLY DAMAGING	1	0	1
rs891087	D261E	BENIGN	0	1	0
rs2162771	P830L	BENIGN	0	1	0
rs13306449	Y1361C	PROBABLY DAMAGING	1	0	1
rs35045353	G811S	BENIGN	0.441	0.89	0.9
rs1051691	I448T	PROBABLY DAMAGING	0.996	0.55	0.98
rs1051692	Y171H	BENIGN	0.024	0.95	0.81
rs2229429	D546E	BENIGN	0.032	0.95	0.82
rs7508518	A2G	BENIGN	0	1	0
rs52800171	W1220L	PROBABLY DAMAGING	1	0	1
rs55816055	S353P	POSSIBLY DAMAGING	0.528	0.88	0.9
rs56395521	L1065V	BENIGN	0	1	0
rs72549237	V362I	BENIGN	0.003	0.98	0.44
rs76077021	R889W	BENIGN	0.111	0.93	0.86
rs76673783	E664G	POSSIBLY DAMAGING	0.592	0.87	0.91
rs78433961	R796S	BENIGN	0.001	0.99	0.15
rs78827745	M65K	POSSIBLY DAMAGING	0.934	0.8	0.94
rs79312957	R413C	PROBABLY DAMAGING	0.999	0.14	0.99
rs113527718	S1297G	BENIGN	0.004	0.97	0.59
rs138528064	T320M	BENIGN	0.199	0.92	0.88
rs140762552	T107M	PROBABLY DAMAGING	1	0	1
rs140852238	E51K	BENIGN	0.003	0.98	0.44
rs141484557	G262S	POSSIBLY DAMAGING	0.939	0.8	0.94
rs142391704	A706D	BENIGN	0	1	0
rs142910337	D75G	BENIGN	0	1	0
rs143523271	S748L	BENIGN	0	1	0

SNP_ID	Mutation	Polyphen Result	Score	Sensitivity	Specificity
rs143919163	G192D	BENIGN	0.005	0.97	0.74
rs144029037	V900I	BENIGN	0.048	0.94	0.83
rs146588336	D946E				
rs147671523	E517G	POSSIBLY DAMAGING	0.726	0.86	0.92
rs148838377	P755S	BENIGN	0	1	0
rs149536206	H8598*	-	-	-	-
rs150114699	L991I	BENIGN	0.442	0.89	0.9
rs181150880	R410Q	POSSIBLY DAMAGING	0.935	0.8	0.94
rs182552223	T858A	BENIGN	0.007	0.96	0.75
rs183360558	D893N	BENIGN	0	1	0
rs185736681	R1053C	BENIGN	0.223	0.91	0.88
rs187282966	R889Q	BENIGN	0.252	0.91	0.88
rs199580495	S1033F	PROBABLY DAMAGING	0.968	0.77	0.95
rs199599404	M1319I	BENIGN	0.007	0.96	0.75
rs199659271	C219R	PROBABLY DAMAGING	1	0	1
rs200059069	K411Q	POSSIBLY DAMAGING	0.75	0.85	0.92
rs200110540	V866I	BENIGN	0	1	0
rs200199169	P271L	BENIGN	0	1	0
rs200400127	A1340V	BENIGN	0.021	0.95	0.8
rs200921389	G1048D	BENIGN	0.009	0.96	0.77
rs201147780	K294R	BENIGN	0.008	0.96	0.76
rs201466857	T858M	PROBABLY DAMAGING	0.994	0.69	0.97
rs201506342	P1312T	POSSIBLY DAMAGING	0.616	0.87	0.91
rs201978448	A537V	BENIGN	0.143	0.92	0.86
rs201979105	S1221A	PROBABLY DAMAGING	0.997	0.41	0.98
rs202160383	R1128H	BENIGN	0.031	0.95	0.82

SNPs were categorized as benign and harmful based on a PSIC score differential. PolyPhen-2 scores range from 0.000 (likely beneficial) to 0.999 (likely harmful). The PSIC values ranged from 1.51 to 3.41, and 21 out of the 57 nsSNPs were predicted to be "damaging." The SIFT (Sorting Intolerant from Tolerant) software identified 18 nsSNPs that were predicted by the PolyPhen service to be harmful. I-Mutant is a routine tool built on a neural network that is used to analyze changes in protein stability by taking into account single-site mutations. Additionally, I-Mutant offers the scores for free energy modifications that were computed using the FOLD-X energy-based web server. About 93% precision can be attained by combining the FOLD-X and I-Mutant calculations. A destabilization threshold for an SNP of about 1.5 Kcal/mol has been taken into consideration. I-Mutant determined 46 nsSNPs to be destabilized based on DDG values (Table 4.3).

Table 4.3: List of nsSNPs stability predicted by I-MUTANT

SNP_ID	Mutation	Stability
rs1799816	V1012M	Decrease
rs52836744	G58R	Decrease
rs121913144	R1027*	-
rs121913145	H236R	Decrease
rs121913156	R1201Q	Decrease
rs891087	D261E	Increase
rs2162771	P830L	Decrease
rs13306449	Y1361C	Increase
rs35045353	G811S	Decrease
rs1051691	I448T	Decrease
rs1051692	Y171H	Decrease
rs2229429	D546E	Increase
rs7508518	A2G	Decrease
rs52800171	W1220L	Decrease
rs55816055	S353P	Increase
rs56395521	L1065V	Decrease
rs72549237	V362I	Decrease
rs76077021	R889W	Decrease
rs76673783	E664G	Decrease
rs78433961	R796S	Decrease
rs78827745	M65K	Decrease
rs79312957	R413C	Decrease
rs113527718	S1297G	Decrease
rs138528064	T320M	Decrease
rs140762552	T107M	Decrease
rs140852238	E51K	Decrease
rs141484557	G262S	Decrease
rs142391704	A706D	Decrease
rs142910337	D75G	Decrease
rs143523271	S748L	Increase
rs143919163	G192D	Decrease
rs144029037	V900I	Decrease
rs146588336	D946E	Increase
rs147671523	E517G	Increase
rs148838377	P755S	Decrease
rs149536206	H8598*	-
rs150114699	L991I	Decrease
rs181150880	R410Q	Decrease
rs182552223	T858A	Decrease
rs183360558	D893N	Decrease
rs185736681	R1053C	Decrease
rs187282966	R889Q	Decrease
rs199580495	S1033F	Increase

SNP_ID	Mutation	Stability
rs199599404	M1319I	Decrease
rs199659271	C219R	Decrease
rs200059069	K411Q	Increase
rs200110540	V866I	Decrease
rs200199169	P271L	Decrease
rs200400127	A1340V	Decrease
rs200921389	G1048D	Decrease
rs201147780	K294R	Increase
rs201466857	T858M	Decrease
rs201506342	P1312T	Decrease
rs201978448	A537V	Increase
rs201979105	S1221A	Decrease
rs202160383	R1128H	Decrease

Finally, we chose 18 significant nsSNPs since I-Mutant analysis revealed lower structural stability and PROVEAN, PolyPhen, and SIFT tools predicted them to be harmful (Table 4.4).

Table 4.4: Common amino acid change of deleterious nsSNPs in human INSR gene predicted by PROVEAN and PolyPhen algorithms.

SNP_ID	Mutation	PROVEAN Result	PROVEAN Score	Polyphen Result	Score
rs52836744	G58R	Deleterious	-6.762	PROBABLY DAMAGING	1
rs121913145	H236R	Deleterious	-5.616	PROBABLY DAMAGING	1
rs121913156	R1201Q	Deleterious	-3.701	PROBABLY DAMAGING	1
rs13306449	Y1361C	Deleterious	-4.157	PROBABLY DAMAGING	1
rs1051691	I448T	Deleterious	-3.774	PROBABLY DAMAGING	0.996
rs52800171	W1220L	Deleterious	-11.648	PROBABLY DAMAGING	1
rs55816055	S353P	Deleterious	-2.761	POSSIBLY DAMAGING	0.528
rs76673783	E664G	Deleterious	-5.068	POSSIBLY DAMAGING	0.592
rs78827745	M65K	Deleterious	-3.808	POSSIBLY DAMAGING	0.934
rs79312957	R413C	Deleterious	-5.623	PROBABLY DAMAGING	0.999
rs140762552	T107M	Deleterious	-2.922	PROBABLY DAMAGING	1
rs141484557	G262S	Deleterious	-3.037	POSSIBLY DAMAGING	0.939
rs147671523	E517G	Deleterious	-4.293	POSSIBLY DAMAGING	0.726
rs199580495	S1033F	Deleterious	-5.642	PROBABLY DAMAGING	0.968
rs199659271	C219R	Deleterious	-9.831	PROBABLY DAMAGING	1
rs201466857	T858M	Deleterious	-3.384	PROBABLY DAMAGING	0.994
rs201506342	P1312T	Deleterious	-3.008	POSSIBLY DAMAGING	0.616
rs201979105	S1221A	Deleterious	-2.698	PROBABLY DAMAGING	0.997

4.3.3 Effects of nsSNPs on Protein Structure

The PDBsum web-based tool from EMBL-EBI was used to search the INSR protein structures. The amino acid sequences of two related protein structures having PDB ID, 2HR7 and 4IBM, were discovered to be 100% identical. Due to necessary maintenance, the single amino acid polymorphism (SAAP) database server (<http://www.bioinf.org.uk/saap/db/>) is not available. As a result, SAAP did not allow us to map the harmful nsSNPs into the protein structure. Through the LS-SNP/PDB service, the harmful nsSNPs were mapped to information on the protein structure. This source states that 4IBM had 4 nsSNPs, while 2HR7 had 9 nsSNPs. The LS-SNP/PDB server additionally forecasts solvent accessibility and conservation ratio for specific protein structures in addition to SNP scanning. Table 4.5 provides a summary of the mapping of mutant structures, their solvent accessibility, and their conservation ratios.

Table 4.5: Mapping of nsSNPs in 2HR7 and 4IBM 3D structures

PDB_ID_2HR7	Mutation	PDB Number	Residue	Solvent Accessibility	Conservation
rs52836744	G58R	31		Intermediate 10%	5%
rs121913145	H236R	209		Intermediate 23%	10%
rs1051691	I448T	421		Buried 1%	1%
rs55816055	S353P	326		Buried 5%	3%
rs78827745	M65K	38		Buried 0%	8%
rs79312957	R413C	386		Exposed 58%	3%
rs140762552	T107M	80		Buried 2%	5%
rs141484557	G262S	235		Exposed 46%	25%
rs199659271	C219R	192		Buried 5%	11%
PDB_ID_4IBM	Mutation	PDB Number	Residue	Solvent Accessibility	Conservation
rs121913156	R1201Q	1174		Intermediate 10%	0%
rs52800171	W1220L	1193		Buried 1%	0%
rs199580495	S1033F	1006		Intermediate 30%	0%
rs201979105	S1221A	1194		Buried 5%	0%

Thirteen of the 18 nsSNPs that PROVEAN or PolyPhen predicted to be harmful were mapped to the natural structures of PDB ID 2HR7 and 4IBM. The PYMOL mutation tool was used to all the functional nsSNPs predicted by the PROVEAN and PolyPhen programs. For comparison with the native structures, a model for each functional nsSNP was created using the PYMOL mutagenesis tool and displayed using the UCSF Chimera tool (Figure 4.2, only mutants rs1051691 (I421T) and rs121913156 (R1174Q) are shown).

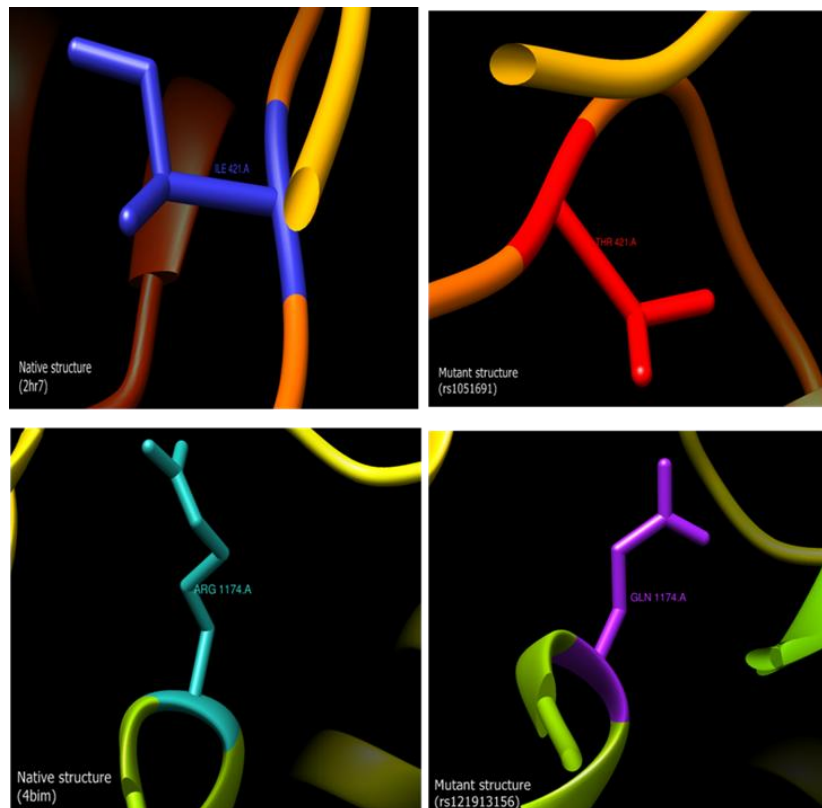


Figure 4.2: A comparison of amino acid substitutions due to nsSNPs. Two mutant structures of deleterious nsSNPsrs1051691 (I421T) and rs121913156 (R1174Q) are compared to their native structures 2hr7 and 4ibim, respectively. Models were generated by using PYMOL and visualized by UCSF Chimera.

Both the native structures (2HR7 and 4IBM) and the mutant-modeled structures are subjected to energy minimization. To save energy, the KoBaMIN web server employs a force field. Table 4.6 summarizes the total energy for all mutant and native models following reduction. The natural structures of 2HR7 and 4IBM have total energies of 22087.6969 kJ/mol and 13041.4646 kJ/mol, respectively. Both the 2HR7 and 4IBM mutant models exhibit a change in total energy caused by mutation. RMSD is a measurement of

how far mutant structures deviate from their original forms. The higher difference between the two structures indicates the greater RMSD value. Functional activity is then impacted by structural alterations. Table 4.6 contains the RMSDs for all the mutant structures.

Table 4.6: RMSD and total energy after energy minimization of native structures and their mutant 3D models

Molecules	RMSD (Å)	Total energy after energy minimization (kJ/mol)
2HR7 native-type structure	6.019	-22087.6969
2HR7 mutant (rs52836744)	6.007	-21968.7347
2HR7 mutant (rs121913145)	5.985	-21815.0585
2HR7 mutant (rs1051691)	5.997	-22160.7304
2HR7 mutant (rs55816055)	5.957	-21903.7516
2HR7 mutant (rs78827745)	5.991	-21816.4353
2HR7 mutant (rs79312957)	6.025	-21962.4576
2HR7 mutant (rs140762552)	5.992	-21823.0448
2HR7 mutant (rs141484557)	5.995	-22076.7362
Molecules	RMSD (Å)	Total energy after energy minimization (kJ/mol)
2HR7 mutant (rs199659271)	5.98	-21736.5417
<i>4IBM native-type structure</i>	0.404	-13041.4646
4IBM mutant (rs121913156)	0.436	-13091.3512
4IBM mutant (rs52800171)	0.39	-12830.5659
4IBM mutant (rs199580495)	0.402	-11940.1628
4IBM mutant (rs201979105)	0.376	-13076.6808

In comparison to native structures, which have RMSD values of 6.019 and 0.404, respectively, the mutants rs79312957 and rs121913156 have higher RMSD values of 6.025 and 0.436, correspondingly. There is a possibility that these two nsSNPs alter the proteins' structural makeup. The PROVEAN and PolyPhen servers also demonstrated the deleteriousness of these two nsSNPs. The 3D model of chain A was overlaid with the 3D structures of the native INSR protein crystal structures of 2HR7 and 4IBM. The overlaid structures demonstrated that the mutations may have significantly altered the structure of the protein and, consequently, its function (Figure 4.3; only rs79312957 is shown).

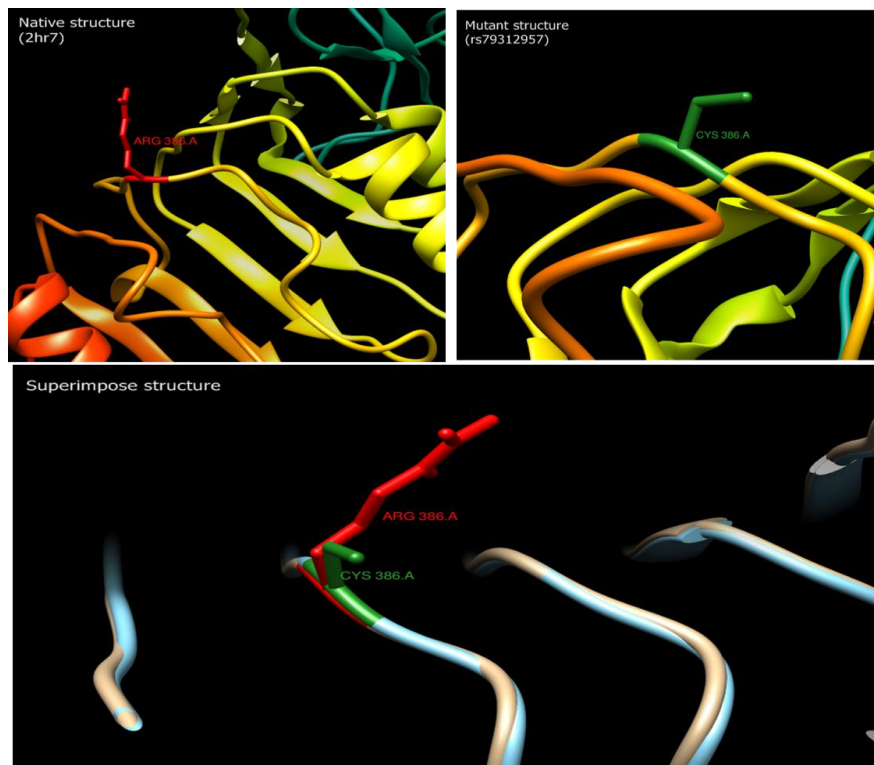


Figure 4.3: Superimposition of native and mutant structures. Native structure (2HR7) shows arginine at position 386 (a) and mutant modeled structure rs79312957 (R386C) shows cysteine residue at the corresponding position (b). (c) shows the superimposition of the native structure

The mutants' substituted amino acid residues may have changed how the INSR conforms, how nearby amino acids network together, or how the substrate and receptor interact (Azad *et al.*, 2012; Azad *et al.*, 2014).

4.3.4 Effects of nsSNP on Protein Stability

The effects of the nsSNPs on protein stability were calculated using FOLD-X by the mCSM server, which determines the Gibbs free energy DDG using an empirical energy equation. The position and kind of a replaced residue are taken into account by the empirical energy terms. The mCSM a structure-based prediction tool. To gather the most data on the impact of single amino acid substitutions, two different analysis protocols were used: (1) all nsSNPs were taken into consideration individually, and their impact on protein stability and interaction potential was determined; (2) nsSNPs were taken into consideration in accordance with allelic sequences. All of the structures were initially reduced in order to get the protein stability value. Then, using FOLD-X 3.0's Build Model function, the structures for each individual amino acid variant were created. Finally, utilizing the complex properties that were examined, it was possible to identify how each individual amino acid alteration affected the protein stability of INSR. When the DDG was greater than 0 or less than 0, the mutation was regarded as destabilizing or stabilizing, accordingly. In order to forecast the mutant structure's protein stability upon mutation, all the mutant structures ultimately produced by the PROVEAN, PolyPhen, and I-Mutant algorithms were eventually uploaded to the mCSM server. All structures were projected by the mCSM to be "Destabilizing," with two of them being "Highly Destabilizing" (Table 4.7).

Table 4.7: Protein stability upon mutation

Molecules	Mutation	PDB Residue Number	RSA (%)	Predicted $\Delta\Delta G$	Outcome
<i>2HR7 native-type structure</i>					
2HR7 mutant (rs52836744)	G58R	31	33.2	-1.11	Destabilizing
2HR7 mutant (rs121913145)	H236R	209	30.5	-0.248	Destabilizing
2HR7 mutant (rs1051691)	I448T	421	0	-2.403	Highly Destabilizing
2HR7 mutant (rs55816055)	S353P	326	15.5	-0.339	Destabilizing
2HR7 mutant (rs78827745)	M65K	38	0	-1.568	Destabilizing
2HR7 mutant (rs79312957)	R413C	386	70.2	-0.931	Destabilizing
2HR7 mutant (rs140762552)	T107M	80	2.3	-0.387	Destabilizing
2HR7 mutant (rs141484557)	G262S	235	70.3	-0.775	Destabilizing
2HR7 mutant (rs199659271)	C219R	192	3.2	-0.039	Destabilizing
<i>4IBM native-type structure</i>					
4IBM mutant (rs121913156)	R1201Q	1174	11.5	-1.347	Destabilizing

4IBM mutant (rs52800171)	W1220L	1193	2.1	-3.223	Highly Destabilizing
4IBM mutant (rs199580495)	S1033F	1006	16.7	-0.888	Destabilizing
4IBM mutant (rs201979105)	S1221A	1194	9.5	-1.858	Destabilizing

This study comes to the conclusion that 13 nsSNPs, particularly rs1051691 and rs52800171, impair protein stability, are intolerable, or may cause function loss. Their inclusion in the INSR raises the risk of diseases caused by the INSR and altered transcriptional and cell cycle control. As a result, it is more likely that they are involved in disease predisposition. Therefore, in order to gather comprehensive information on their consequences, these mutations should be prioritized for future investigation. The actual structures should be identified by X-ray crystallography or nuclear magnetic resonance spectroscopy in order to confirm the structures hypothesized in this study.

CHAPTER FIVE

Analysis of Single Nucleotide Polymorphism in Insulin Receptor in Diabetic Subjects

Summary

To investigate the impact of the SNPs in the human INSR gene, we analyzed the insulin receptor gene sequences and predicted the deleterious mutation in the insulin receptor using different bioinformatics tools. The findings showed that two mutations in I448T and W1220L located in exon 6 and 21 respectively were highly destabilizing. Their presence in the INSR increases the possibility of altered transcriptional and cell cycle regulation and INSR mediated diseases. To confirm the mutation, we isolated the genomic DNA from patients with Type 2 (non-insulin-dependent) diabetes and without diabetes. Using the gene specific primers, we performed the PCR amplification. The PCR products were purified and sequenced. The sequence was analyzed for the presence of mutation in INSR of study subjects. No SNPs were observed in the exons of 6, 20 and 21 of diabetic patients. However, three SNPs were observed in the exon 11. These mutations in insulin receptor may progress the disease pathogenesis or suppress the disease.

5.1 Introduction

The T2DM has been considerably increased in many regions of the world. IR or metabolic syndrome bear biochemical and physiological disorder of metabolic abnormalities which include hyperinsulinemia, glucose intolerance, increased low-density lipoprotein (LDL), decreased high-density lipoprotein (HDL), and hypertension, and plays an important role in the intimation of the atherosclerotic process (Zhou *et al.*, 2018)). Mutations disrupt insulin responses by decreasing the number of insulin receptors or by reducing the receptor's capability to bind insulin (Boucher *et al.*, 2014)). Insulin stimulates endothelial production of nitric oxide (NO), a vasodilator, which restricts vascular smooth muscle cell growth and also releases a strong vasoconstrictor, endothelin ET-1 (Hartge *et al.*, 2007). This dual action of insulin is mediated by two major signaling pathways. Under physiological conditions, a vasoprotective phosphoinoside-3-kinase (PI3-K)/Akt pathway predominates and is liable for manifestation and activation of endothelial nitric oxide synthase (eNOS). When IR appears, mitogen-activated protein kinase (MAPK) signaling pathway secures which is also linked with endothelial cells by mediating secretion of ET-1

(Olver *et al.*, 2019). Insulin is a very well-studied polypeptide hormone produced by the β -cells of the pancreases in response to nutritional stimuli. Insulin receptor substrate (IRS)-1 is 2-fold more condensed in the intracellular membrane (IM) compartment than in cytosol, whereas IRS-2 is twofold more concentrated in cytosol than in IM. Insulin stimulation induces rapid tyrosine phosphorylation of both IRS-1 and IRS-2. This occurs mainly in the IM compartment. IRS-2 is located predominately in cytosol (Neff *et al.*, 2020). IRS tyrosine phosphorylation is mandatory for insulin response, but depending on which serine is phosphorylated, IRS increases or decreases insulin action (Giraud *et al.*, 2004). Insulin is a very well-studied polypeptide hormone produced by the β -cells of the pancreases in response to nutritional stimuli. In muscle and adiposites, insulin-stimulated glucose uptake is achieved by the translocation of the insulin-sensitive glucose transporter (GLUT-4) from intracellular storage vesicles to the cell surface (Usui *et al.*, 2003). Insulin receptor is a member of the ligand-activated receptor and tyrosine kinase family of transmembrane signaling proteins that collectively are fundamentally important regulators of cell differentiation, growth, and metabolism (Eichler *et al.*, 2016). The enzymes involved in the regulation of glucose metabolism by insulin appear to be regulated by phosphorylation and dephosphorylation on serine and/or threonine residues (Scapin *et al.*, 2018). It is an incompletely resolved issue by which mechanism the tyrosine-specific kinase activity of the insulin receptor can activate serine- and/or threonine-specific phosphorylation. Glucose hemostasis may also be regulated by alteration in glycogen metabolism by transcriptional regulation of phosphoenolpyruvate carboxykinase, the rate-limiting enzyme in gluconeogenesis (Hubbard *et al.*, 1993). Transcription of this enzyme is rapidly decreased after insulin treatment, thus lowering protein levels and leading to diminished glucose synthesis. Insulin was the first peptide hormone to be analyzed in receptor binding studies. Recently, molecular models of ligand binding to receptor have been claimed. The exact regions of the receptor that directly contact hormone remain incompletely defined and are still the object of much scrutiny. The two general experimental approaches are being employed to define ligand-receptor contact regions, affinity labeling and mutagenesis (Chiu *et al.*, 2010). Some researchers identified mutations in the insulin receptor, in PI3K, in the liver glucokinase promoter, GLUT4, in the glycogen synthase, and in the protein phosphatase-1 are responsible for diabetes mellitus (Moller *et al.*, 1990 & Anderson *et al.*, 1992). Consumption of excess calories causes increased visceral fat mass and central obesity is linked to IR (Lee & Pilch, 1994; Purushottam *et al.*, 2019). In addition, lipid

accumulation, lipotoxicity, glucotoxicity, and the secretion of inflammatory markers are linked to the development of local and systemic IR (D'Alessandris *et al.*, 2004). The pathogenesis of IR can be grouped into: genetic defects, adipose dysfunction, physical inactivity, obesity, and inflammation. Insulin receptor is a big warehouse of diseases such as DM, one of the most dangerous diseases in human beings. Any change or mutation in insulin receptors may change disease pathogenesis. SNPs consist of a single change in the DNA code. Variations in the DNA sequences of humans can affect how humans develop diseases and respond to pathogens, chemicals, medication, vaccines, and other agents. SNPs may fall within coding sequences of genes, non-coding regions of genes, or in the intergenic regions between genes. SNPs that are not in protein-coding regions may still have consequences for gene splicing, transcription factor binding, or the sequence of non-coding ribonucleic acid (RNA). SNPs in coding region may alter the structural conformation of the protein, its stability and the intramolecular and intermolecular networks (Azad *et al.*, 2012, Mahmud *et al.*, 2016). We have reported benign, destabilizing and highly destabilizing SNPs in the INSR having the PDB ID, 2HR7 and 4IBM (Mahmud *et al.*, 2016). However, there are variations between human populations, so an SNP allele that is common in one geographical or ethnic group may be much rarer in another. Therefore, the SNPs in the INSR of the diabetic patients in Bangladesh may differ from those in that of the database. Hence, the SNPs in some of the exons of the human INSR gene in diabetic and non-diabetic patients have been investigated.

5.2 Materials and Methods

Methodologies used in this chapter are outlined in figure 5.1.

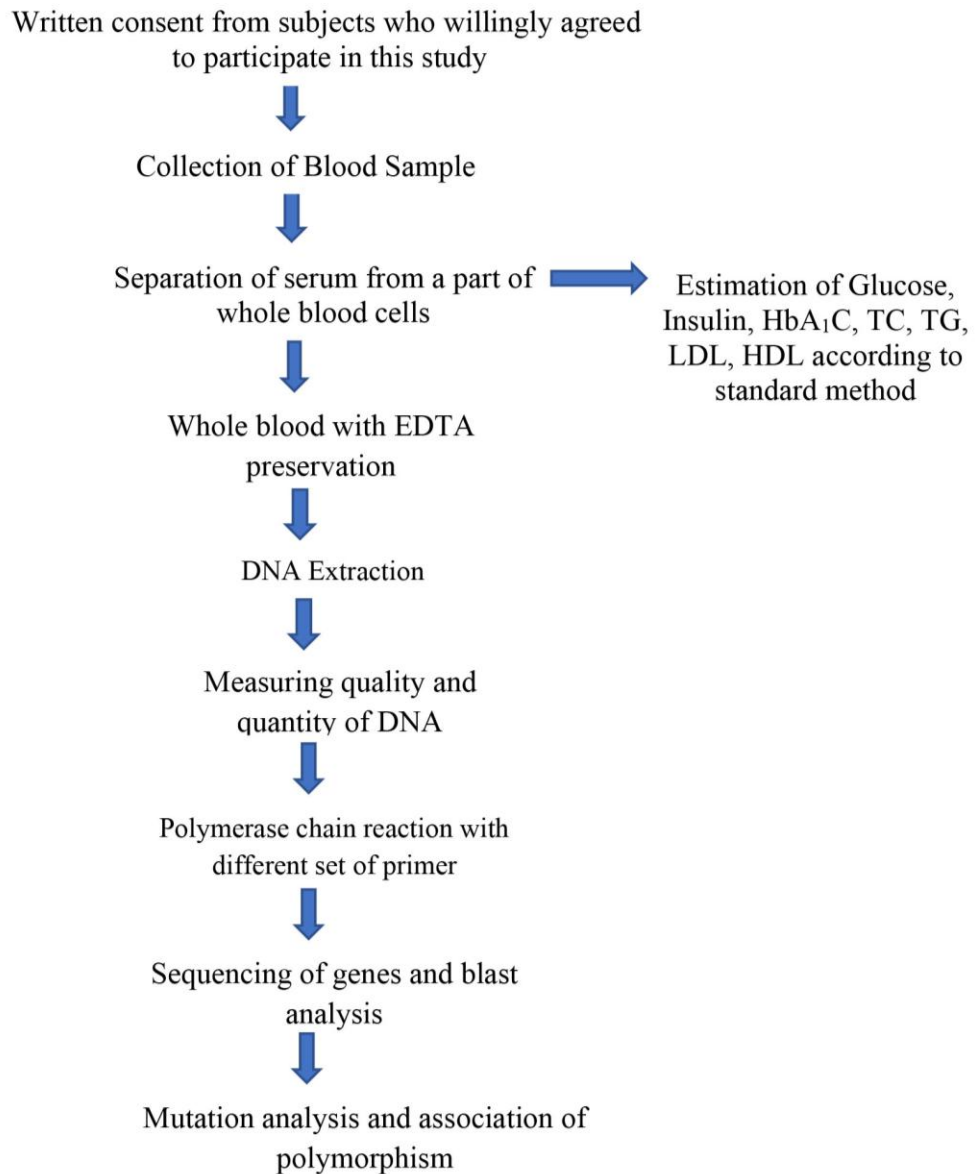


Figure 5.1: A flow chart of methodologies used in this chapter.

5.2.1 Isolation of DNA from Blood

Blood samples were collected from diabetic and non-diabetic subjects. Genomic DNA was extracted from blood samples by using genomic DNA isolation kit (FavorPrep™, Favorgen, Taiwan) according to the instructions of manufacturer. The study was approved by Institutional Ethical Review Board of North East Medical College Hospital, Sylhet, and all subjects provided signed informed consent in accordance with our guidelines for the protection of human subjects. Nanodrop spectrophotometer was used to determine the DNA quality and quantity. The absorbance ratios (A_{260}/A_{280}) of the

extracted DNA samples ranged from 1.8 -2.0 was considered as the criteria for good-quality DNA. Furthermore, the integrity of the genomic DNA was investigated upon agarose gel electrophoresis.

5.2.2 PCR Mediated Amplifications of the Exons in the INSR Gene

PCR was carried out to amplify the target exons in the genomic DNA. Exon sequence-specific primers for Exon-6, Exon-11, Exon-20 and Exon-21 used in this study have been shown in table 5.1.

Table 5.1: Pairs of primers for amplifying different exons of the INSR gene

Primer	Forward	Reverse	size
Exon-6	5'AGGCACGTAGCACTGAACA	5'TGTAATGCACTTGAATCATGCTG	433
Exon-11	5'GTGGTCTGTCTAATGAAGTT	5'GAATTGGTGAAGCATCTGCT	238
Exon-20	5'AGGTTAAGAGCGTGTGAACCT	5'GAATTCAAGCCCAGCGTCCAT	208
Exon-21	5'TGTTACTACTATCAACTGTC	5'ACCTGTAACATACAGCATGC	291

The PCR (1 cycle at 95 °C for 2 minutes for denaturation of DNA; 35 cycles at 95 °C for 30 seconds, at 55 °C for 30 seconds and at 72 °C for 1 minute; 1 cycle at 72 °C for 10 minutes) was performed with 25 µl reaction mixture (1µl template DNA, 1 kb DNA ladder, dNTPs, Primers, Agarose, Ethidium bromide).

5.2.3 PCR Products Purification and DNA Sequencing

To get rid of any unused dNTPs primers, and other components, we purified the amplified PCR products after gel electrophoresis. These impurities have the potential to interfere with sequencing reactions and result in false picks. The successful purification of the PCR products is shown in (Figure 5.2-5.5). The purified DNA samples were sequenced by Sanger method with corresponding forward and reverse primers.

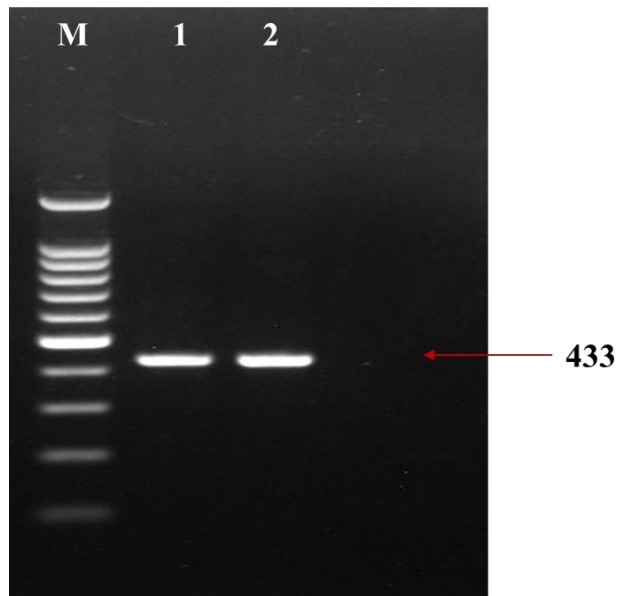


Figure 5.2: DNA bands of exon 6 after gel purification. Lane M: Molecular marker (1 kb DNA ladder, Promega Corporation); Lanes 1 & 2 show the purified PCR product of exon 6.

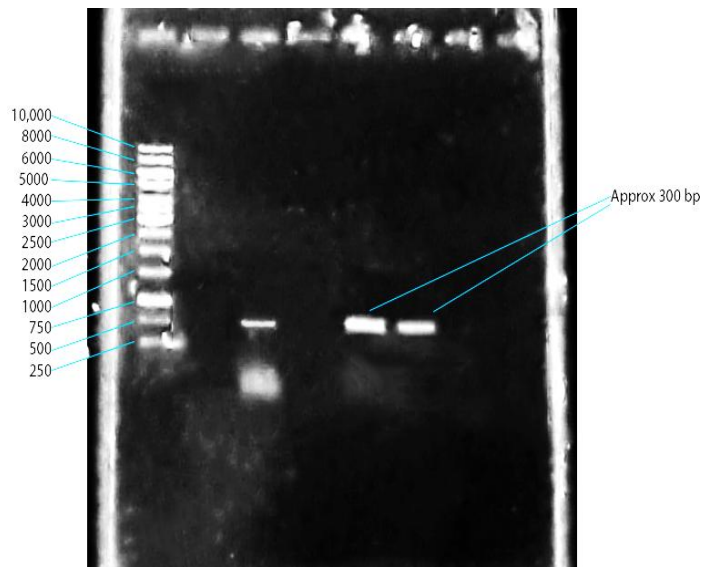


Figure 5.3: DNA bands of exon 11 after gel purification. First lane: Molecular marker (1 kb DNA ladder, Promega Corporation); the indicated bands represent the purified PCR product of exon 11.

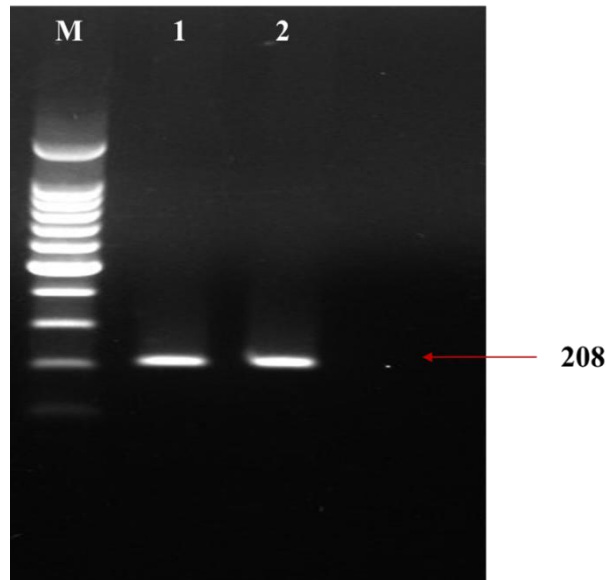


Figure 5.4: DNA bands of exon 20 after gel purification. Lane M: Molecular marker (1 kb DNA ladder, Promega Corporation); Lanes 1 & 2 show the purified PCR product of exon 20.

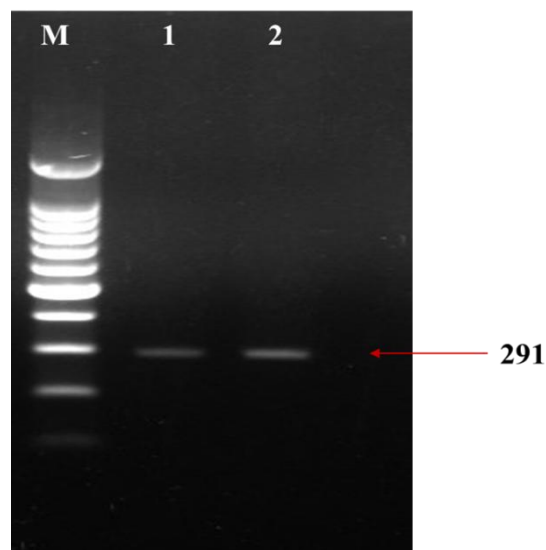


Figure 5.5: DNA bands of exon 21 after gel purification. Lane M: Molecular marker (1 kb DNA ladder, Promega Corporation); Lanes 1 & 2 show the purified PCR product of exon 21.

5.2.4 Bioinformatics Analysis of DNA Sequence for Prediction of SNPs in INSR

BioEdit 7.2 tool was used to open the raw file of DNA sequences of PCR products. A consensus sequence was confirmed from the sequence done with the forward and the

reverse primers. The consensus DNA sequences of PCR products aforementioned were used to the BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), a computer-based similarity search tool. The similar sequences were retrieved for pairwise or multiple alignments. The pairwise alignment was performed with EMBOSS Needle (https://www.ebi.ac.uk/Tools/psa/emboss_needle/) and the multiple alignment was done with CLUSTAL Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). The both alignments were employed for predicting the SNPs in INSR.

5.2.5 Mutation Analysis and Homology Modeling

Mutation Surveyor software version 5.0 (<https://softgenetics.com/products/mutation-surveyor/>) was used for the detection of mutations in the INSR. The 3D models of both native and mutant sequences of INSR were constructed by Swiss model (<https://swissmodel.expasy.org/>). All the models were viewed with PyMol (<https://pymol.org/2/>) for further analysis such as superimposition and visualization.

5.3 Results and Discussion

5.3.1 Generation of Consensus Sequence

The PCR products obtained using both forward and reverse primers, were sequenced by Sanger method. The output of the DNA sequence file (ab1) provided by the sequencing Service Provider was opened in BioEdit 7.2 tool (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>) for generating consensus sequences from the sequences obtained by using forward and reverse primers. Both forward and reverse sequence files were opened in this software and a reverse complement of the reverse sequence was generated. Then pairwise alignment of both sequences was done and a consensus sequence was created. The generated sequence was used to BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) so that the similar sequences are found from the NCBI database (Figure 5.6).

Pairwise alignment between sequence of diabetic patients (query sequence) and highest similar sequence found after BLAST

Reverse DB:

Homo sapiens insulin receptor (INSR) gene, complete cds
 Sequence ID: [AH002851.2](#) Length: 14767 Number of Matches: 1

Range 1: 13459 to 13703 [GenBank](#) [Graphics](#) ▼ Next Match ▲ Previous Match

Score	Expect	Identical	Gaps	Strand
-403 bits(218)	2e-108	236/245(96%)	0/245(0%)	Plus/Plus
Query 1	TTOTTACTACTATCAACTGTCATCGGCAGG	GGCGTGGTCCCTTTGGGAAATCCCC	60	
Sbjct 13459	TTOTTACTACTATCAACTGTCATCGGCAGG	GGCGTGGTCCCTTTGGGAAATCACC	13518	
Query 41	AGCTTGGCAGAACAGGCTTACCCAAAGGCTGTCTAACGAAACAGGTCCTTGAAATCCGTCATG	120		
Sbjct 13519	AGCTTGGCAGAACAGGCTTACCCAAAGGCTGTCTAACGAAACAGGTCCTTGAAATTTGTTCATG	13578		
Query 121	GATGGAGGGGTATCTGGATCCACCCGACAACTGTCCAGAGAGAGTGTAAAGTCAGAAAAGG	180		
Sbjct 13579	GATGGAGGGGTATCTGGATCCACCCGACAACTGTCCAGAGAGAGTGTAAAGTGTAGAAAAGG	13638		
Query 181	TTTAAGCTGTGTGAGGTGTTCCGTTGAAAAGGGTATTGCCCTTTACACGTGTCTTGGTTTT	240		
Sbjct 13639	TTTAAGGTGTGTGAGGTGTTCCGTTGAAAAGGGTATTGCCCTTTACACGTGTGCTTGGTTTT	13698		
Query 241	GCCTT	245		
Sbjct 13699	GCCTT	13703		

Figure 5.6: BLASTn results of the consensus sequence generated from exon 11.

The similarity search by BLAST showed that the sequences of exon 6, 20 and 21 are 100% identical with those in the native INSR. However, the sequence of exon 11 showed 98.92% identity and 96% query coverage with the sequence of exon 11 in the native INSR. Therefore, further analyses were done only with the exon 11.

5.3.2. Mutation in the INSR of Diabetic Patient

After sequencing, nucleotide sequence was converted into a peptide sequence using different tools and we compared it with normal patient INSR. In the case of exon 11, we found three mutations including threonine to proline, phenylalanine to serine, and glutamate to proline (Figure 5.7).

Mutational Analysis

Three mutation was found in exon-11 of insulin receptor indicated in red color

Amino Acid Seq:

```
NGTGGRRGAAAAPLLVAVAAALLLQAAAGHLYPGEVCPGMDIKNHLTRLHELENCSEVIEGHLQIILLMFKTRP  
RDFADLSEFFKRLIMITDYLLLFVYGLSELSKDLFPNLTVINGSRLEFFVYALVIKFMVHLKLEGLVYHLMNIT  
RGEVRIERHNEELCYLATIDWBRILDSVEENYIVLNRDDHRECCGDIQFOTARGGTTCNPATVINGGQFVBRGW  
THSHCCQKVOPTICKSHGCTAEGLCCHSECLGHCQSDPDPFTKCVACRHFYLDGRCVETCFYKHPQDWRC  
VNRFFCQDLHRECKNREBQCCHQYVIRHNKCIPKCSQYTHSSRLLCTFCLOGRCFKVCHLLREKTIQD  
VTSAGELRGCTVINGSLIINIRGGNMLAAELEANLQLEIEISGVYKIRRSYALVSLSPFPKRLRLRQETL  
EIGHYSEFYALDNQNLRLQLDNWSKHNHLYITQGKLFPHYHFKLCLSEIKHMEYVSGTSGRQERNDIALKTHG  
DANCFEELKFDYIKTSFDKILLRNEPYNRPDDELGFHLYKEARYQNVTEFQSQDACCGRNRVVD  
IDPFLRSDNDFKSCQNDIFGNLMRGLKFPWTOVAIEVKTILVTFSDSRRTYGAKSDIIVVQTDATNPEVFLDIE  
VNSSSQIILKWKPPSPDPNGNITHYLVFWERQAEDESELFFELDYCLKGLKLPRTWSPFPFESDSSQKHNQD  
EYDSSAGECCSCPKTDSQILKLELESDFPKCFEDYLHNVVVYFRKTSSTGTGAEDPFPSSKRLSLGDAQNV  
TVAVFTVAAPFTSTGUTSFSRSHAPFKAVPKRELVISGLRHFQYRIEIQACHQCFPFRKCPALAV  
SATTMPEAKADDIVGFPVTHEIFENNVVHLWQSPKRPNGLIIVLYEVSYRRYGQDEKELHLCVSRKHPALSRG  
CRLRQLSPGNYSVRIKATSLAGNGGDFTEPTVYVYEDYLEDVPSNIAKIIIGPLIFVFLFQVYVIGSIVLFLK  
KQDFDGLDGLYASNSFEYLSASDVFPQSVVYDWRWVSRKMITLRLPELQGSFGHVEGDAKDIKKA  
ETRVAVRTVNESASLRERIEFLNKASVMKGFCTCHNVVRLLGVVSKGQFTLVVMELMAGDCLKETLRLR  
EAEHNFQRPFPFTLQEMIQMAAEIADGMAYLNAPKPVRRDLAARNCHVAHDFTVKIQCFPSTRDILITLY  
RKGKGLLFPVRWMAFESLKGQVFTSSDWRBEGVVKRTPAAEQPYQGLSHEQVAKFVLDGGYLDQPH  
SEGVYVLEIIPKIAEQPYQGLSHEQVAKFVLDGGYLDQPH
```

```
CFERVTDLHRMCRQFNPKMRPTFLEIVNLLKODLHPSTFFRVSFFHSEENKAPFESKELENEFKEMENVFLD  
CFER  
RSSHCQREAEAGGRDGGSSLGFKASVEKRIPTYTHNGGKRNRIITLFRSNFS
```

Figure 5.7: Three mutations threonine to proline, phenylalanine to serine, glutamate to proline in exon 11 of INSR gene.

Swiss-model (<https://swissmodel.expasy.org/>), a deep learning-based modeling approach, was applied to construct 3D models of both native and mutant proteins where native protein sequence retrieved from the UniprotKB (P06213). The generated models were further refined by GalaxyRefine, and then the energy minimization was done via Swiss-PdbViewer. Figures illustrate the modeled tertiary structures of human insulin, superimposition visualization of the protein done via PyMol (Figure 5.8a and 5.8b). The red-colored mutations in exon 11 indicate mutation on the diabetic patient (Figure 5.8b).

Homology Modelling of Insulin Receptor

Homology Modelling of Insulin Receptor by SWISS Modeling

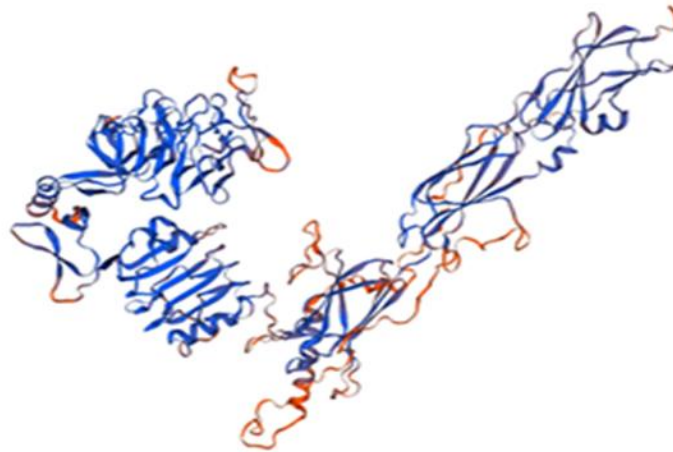


Figure 5.8(a): 3D model of INSR

Superposition of Insulin Receptor

Superposition of Insulin Receptor by PyMol
Exon-11 mutation is indicated by red sign

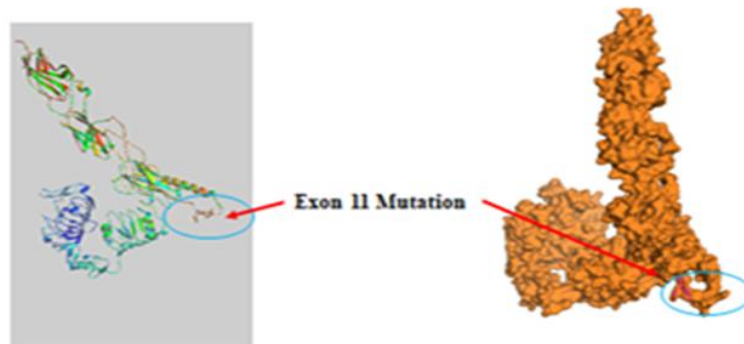


Figure 5.8(b): 3D structure analysis of the INSR. The Swiss-model was used to construct the 3D structure, and these structures were visualized by PyMol

Mammalian cells use glutamine to feed the tricarboxylic acid (TCA) cycle as an alternative source of carbon, and a precursor for proteins, lipids, and nucleic acids. Glutamine is also a key precursor in the synthesis of the antioxidant glutathione, which is important in maintaining the redox balance in cells and tissues. Glutamine can be converted to proline via glutamate. Proline metabolism has complex roles in a variety of biological processes, including cell signaling, stress protection, and energy production. Proline also contributes to

the pathogenesis of various disease-causing organisms. Serine plays an important role in cell signaling. Serine participates in the biosynthesis of biomolecules such as amino acids, nucleotides, phospholipids, and sphingolipids. Serine is needed for the proper metabolism of fats and fatty acids. It also helps in the production of antibodies. However, the effects of these mutations on INSR, the biological activities and the physiological importance of these events remain uncertain. The single amino acid substitution in the exon 11 of the INSR does not significantly change the intramolecular interaction or hydrogen bonding network. Consequently, the structural conformation of the INSR remains almost unchanged (Figure 5.9). Therefore, future research work is necessary to determine which elements are disturbed in the insulin resistant state. Although much work has been done to elucidate the complex pathogenesis of INSR, more human studies are still needed.

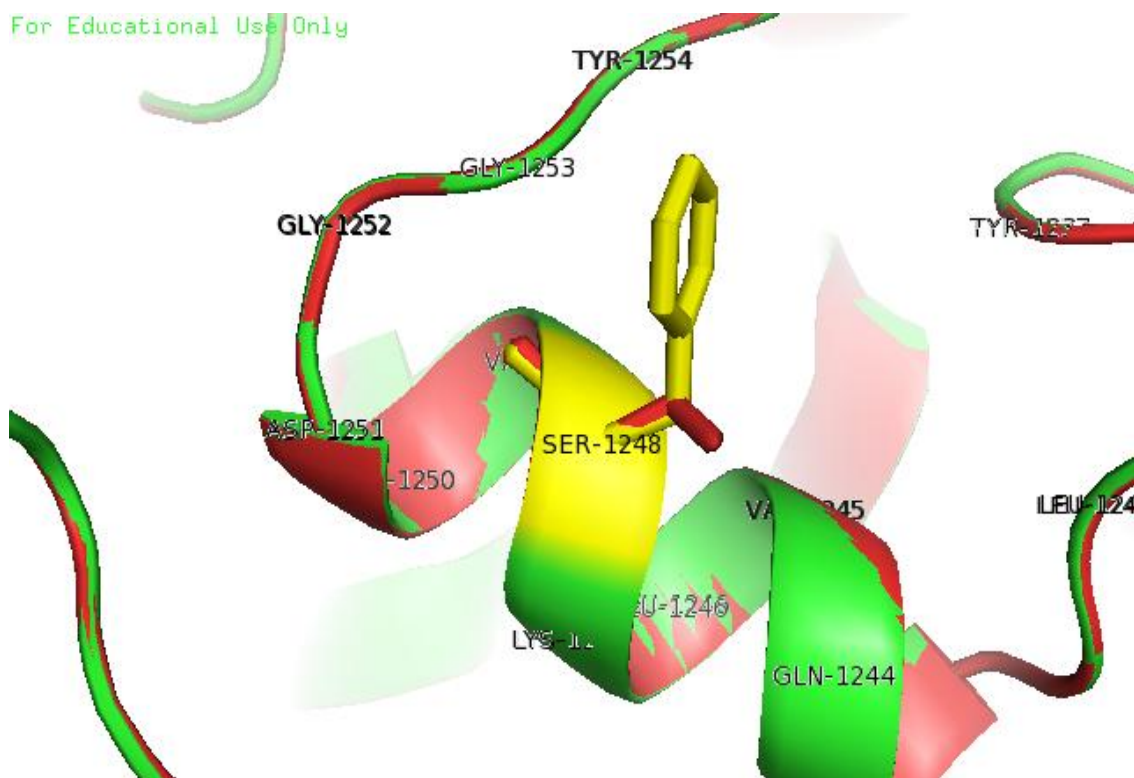


Figure 5.9: The structural conformation of the INSR showing the mutant portion of the exon 11. The 3D model of the mutant exon 11 (green) was superposed with the 3D structure of the wild exon 11.

5.5 Outcomes of the Research

Findings observed in this chapter support the notion that exon 11 in the INSR in the diabetic subjects included in the present study has three mutations. *In silico* 3D model analysis of these mutations showed no significant changes in the hydrogen bonding networking among the intramolecular amino acid residues. Therefore, the impacts of these mutations in the exon 11 should be analyzed by further study with structural analysis. However, no mutation was observed in exons 6, 20 and 21 of INSR in diabetic patients include herein.

Conclusion

Several disorders, including insulin-resistant syndromes including Leprechaunism, Rabson-Mendenhall syndrome, and type A insulin resistance, are shown to be correlated with SNPs in the INSR gene in this study. This study comes to the conclusion that 13 nsSNPs, particularly rs1051691 and rs52800171, impair protein stability, are intolerable, or may cause function loss. Their inclusion in the INSR raises the risk of diseases caused by the INSR and altered transcriptional and cell cycle control. As a result, it is more likely that they are involved in disease predisposition. This study will assist us in assessing the relationship between obesity, dyslipidemia, and T2DM in Bangladeshi population. As far it is known, no research has been done on genetic variations in the prevalence of T2DM in the Bangladeshi community. The Bangladeshi community is more vulnerable to the escalation of T2DM issues, according to the findings of numerous research studies. The Bangladeshi healthcare system is severely plagued by increased prevalence. The identification of the gene polymorphism that increases susceptibility to T2DM will allow for the implementation of prophylactic and preventive interventions. We can demonstrate the metabolic and circulatory consequences of T2DM if we can carry out more studies on T2DM carrying polymorphic variations. The development of personalized treatment will be facilitated by our growing understanding of gene variations and their hazards. As a result, the financial burden on society will decrease.

References

- Abbaspour, N., Hurrell, R., & Kelishadi, R. (2014) Review on iron and its importance for human health. *J Res Med Sci*, 19(2):164–174.
- Abuhendi, N., Qush, A., Naji, F., Abunada, H., Al Buainain, R., Shi, Z., & Zayed, H. (2019) Genetic polymorphisms associated with type 2 diabetes in the Arab world: a systematic review and meta-analysis. *Diabetes Res Clin Pract*, 151:198–208.
- Abagyan, R & Totrov, M. (1994) Biased probability Monte Carlo conformational searches and electrostatic calculations for peptides and proteins, *J Mol Biol*, 235(3):983–1002.
- Albegali, A. A., Shahzad, M., Ullah, M. I., Mahmood, S., & Rashid, M. (2019) Association of genetic polymorphism of PC-1 gene (rs1044498 Lys121Gln) with insulin-resistant type 2 diabetes mellitus in Punjabi Population of Pakistan. *Mol Genet Genomic Med*, 7(8): e775. doi:10.1002/mgg3.775.
- American Diabetes Association (2020) ADA How COVID-19 Impacts People with Diabetes. Available at: <https://www.diabetes.org/coronavirus-covid-19/how-coronavirus-impacts-people-with-diabetes>.
- Andersen, A. S., Kjeldsen, T., Wiberg, F. C., Vissing, H., Schaffer, L., Rasmussen, J. S., & Moller, N. P. (1992) Identification of determinants that confer ligand specificity on the insulin receptor. *J Biol Chem*, 267(19):13681–13686.
- Apicella, M., Campopiano, M.C., Mantuano, M., Mazoni, L., Coppelli, A., & Del Prato S. (2020) COVID-19 in people with diabetes: understanding the reasons for worse outcomes. *Lancet*, 1;8(9):782–92.
- Avogaro, A., Albiero, M., Menegazzo, L., de Kreutzenberg, S., & Fadini, G. P. (2011) Endothelial dysfunction in diabetes: the role of reparatory mechanisms. *Diabetes Care*, 34 (2):285-290. doi:10.2337/dc11-s239.
- Awad, S. F., Al-Mawali, A., Al-Lawati, J. A., Morsi, M., Critchley, J. A., & Abu-Raddad, L. J. (2021) Forecasting the type 2 diabetes mellitus epidemic and the role of key

- risk factors in Oman up to 2050: Mathematical modeling analyses. *J Diabetes Investig*, 12(7):1162–1174. doi:10.1111/jdi.13452.
- Barreiro, L. B., Laval, G., Quach, H., Patin, E., & Quintana Murci, L. (2008) Natural selection has driven population differentiation in modern humans, *Nature Genetics*, 40(3):340–345.
- Barham, D & Trinder, P. (1972) An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst*, 97:142–145.
- Belfiore, A., Malaguarnera, R., Vella, V., Lawrence, M. C., Sciacca, L., Frasca, F., & Vigneri, R. (2017) Insulin Receptor Isoforms in Physiology and Disease: An Updated View. *Endocr Rev*, 38(5):379–431. doi:10.1210/er.2017-00073.
- Berger, C., & Zdzienbło, D. (2020) Glucose transporters in pancreatic islets. *Pflugers Arch*, 472(9):1249–1272. doi:10.1007/s00424-020-02383-4.
- Beg, M., Abdullah, N., Thowfeik, F. S., Altorki, N. K., & McGraw, T. E. (2017) Distinct Akt phosphorylation states are required for insulin regulated Glut4 and Glut1-mediated glucose uptake. *Elife*, 7(6): e26896. doi:10.7554/eLife.26896.
- Bhardwaj, S., Misra, A., Misra, R., Goel, K., Bhatt, S. P., Rastogi, K., & Gulati, S. (2011) High prevalence of abdominal, intra-abdominal and subcutaneous adiposity and clustering of risk factors among urban Asian Indians in North India. *PLoS One*, 6(9): e24362. doi:10.1371/journal.pone.0024362.
- Bilen, O., Kamal, A., & Virani, S. S. (2016) Lipoprotein abnormalities in South Asians and its association with cardiovascular disease: Current state and future directions. *World J Cardiol*, 8(3):247–257. doi:10.4330/wjc.v8.i3.247.
- Borg, M. L., Massart, J., De Castro Barbosa, T., Archilla-Ortega, A., Smith, J. A. B., Lanner, J. T., & Zierath, J. R. (2021) Modified UCN2 peptide treatment improves skeletal muscle mass and function in mouse models of obesity-induced insulin resistance. *J Cachexia Sarcopenia Muscle*, 12(5):1232–1248. doi:10.1002/jcsm.12746.

- Boucher, J., Kleinridders, A., & Kahn, C. R. (2014) Insulin receptor signaling in normal and insulin-resistant states. *Cold Spring Harb Perspect Biol*, 6(1): a009191. doi: 10.1101/cshperspect.a009191.
- Blazquez, E., Velazquez, E., Hurtado-Carneiro, V., & Ruiz-Albusac, J. M. (2014) Insulin in the brain: its pathophysiological implications for States related with central insulin resistance, type 2 diabetes and Alzheimer's disease. *Front Endocrinol (Lausanne)*, 5:161. doi:10.3389/fendo.2014.00161.
- Bohn, M. K., Lippi G, Horvath A, Sethi S, Koch D, Ferrari M, Wang CB, Mancini N, Steele S, & Adeli K. (2020) Molecular, serological, and biochemical diagnosis and monitoring of COVID-19: IFCC taskforce evaluation of the latest evidence. *Clin Chem Lab Med*, 58(7):1037–1052 doi:10.1515/cclm-2020-0722.
- Bremer, A. A., & Jialal I. (2013) Adipose tissue dysfunction in nascent metabolic syndrome. *J Obes*, 2013:393192. doi: 10.1155/2013/393192.
- Cao, X. (2020) COVID-19: immunopathology and its implications for therapy. *Nat Rev Immunol*, 20(5):269–270. doi:10.1038/s41577-020-0308-3.
- Cai, Q., Huang, D., Yu, H., Zhu, Z., Xia, Z., & Su, Y. (2020) COVID-19: abnormal liver function tests. *J Hepatol*, 73(3):566574. doi:10.1016/j.jhep.2020.04.006.
- Cariou, B., Hadjadj, S., Wargny, M., Pichelin, M., Al-Salameh, A., & Allix, I. CORONADO investigators. (2020) Phenotypic characteristics and prognosis of inpatients with COVID-19 and diabetes: the CORONADO study. *Diabetologia*, 63(8):1500–1515. doi:10.1007/s00125-020-05180-x.
- Carter, L. J., Garner, L. V., Smoot, J. W., Li, Y., Zhou, Q., & Saveson, C. J. (2020) Assay Techniques and Test Development for COVID-19 Diagnosis. *ACS central science*, 6(5):591–605. doi:10.1021/acscentsci.0c00501.
- Casadevall, A., & Pirofski, L. A. (2020) The convalescent sera option for containing COVID-19. *J Clin Invest*, 130(4):1545–1548 doi:10.1172/jci138003.
- Chen, X., Stein, T. P., Steer, R. A., & Scholl, T. O. (2019) Individual free fatty acids have unique associations with inflammatory biomarkers, insulin resistance and insulin

- secretion in healthy and gestational diabetic pregnant women. *BMJ Open Diabetes Res Care*, 7(1): e000632. doi:10.1136/bmjdr-2018-000632.
- Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., Qiu, Y., Wang, J., Liu, Y., & Wei, Y. (2020) Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*, 395(10223):507–513. doi:10.1016/S0140-6736(20)30211.
- Chadt, A., & Al-Hasani, H. (2020) Glucose transporters in adipose tissue, liver, and skeletal muscle in metabolic health and disease. *Pflugers Arch*, 472(9):1273–1298. doi:10.1007/s00424-020-02417-x.
- Chait, A., & den Hartigh, L. J. (2020) Adipose Tissue Distribution, Inflammation and Its Metabolic Consequences, Including Diabetes and Cardiovascular Disease. *Front Cardiovasc Med*, 7:22. doi:10.3389/fcvm.2020.00022.
- Chang-Chen, K. J., Mullur, R., & Bernal-Mizrachi, E. (2008) Beta-cell failure as a complication of diabetes. *Rev Endocr Metab Disord*, 9(4):329–343. doi:10.1007/s11154-008-9101-5.
- Choi, C. S., Fillmore, J. J., Kim, J. K., Liu, Z. X., Kim, S., Collier, E. F., & Shulman, G. I. (2007) Overexpression of uncoupling protein 3 in skeletal muscle protects against fat-induced insulin resistance. *J Clin Invest*, 117(7):1995–2003. doi:10.1172/JCI13579.
- Choi, S. M., Tucker, D. F., Gross, D. N., Easton, R. M., DiPilato, L. M., Dean, A. S., & Birnbaum, M. J. (2010) Insulin regulates adipocyte lipolysis via an Akt-independent signaling pathway. *Mol Cell Biol*, 30(21):5009–5020. doi:10.1128/MCB.00797-10.
- Choi, Y., Sims, G. E., Murphy, S., Miller, J. R., & Chan, A. P. (2012) Predicting the functional effect of amino acid substitutions and Indels, *PLoS ONE*, 7(10):e46688. doi: 10.1371/journal.pone.0046688.
- Crafa, A., Calogero, A. E., Cannarella, R., Mongioi, L. M., Condorelli, R. A., Greco, E. A., Aversa, A., & La Vignera, S. (2021) The burden of hormonal disorders: a

- worldwide overview with a particular look in Italy. *Front Endocrinol*, 16(12):694325. doi: 10.3389/fendo.2021.694325.
- Chiu, S. L., & Cline, H. T. (2010) Insulin receptor signaling in the development of neuronal structure and function. *Neural Dev*, 5(7):1–18. doi: 10.1186/1749-8104-5-7.
- Copps, K. D., & White, M. F. (2012) Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2. *Diabetologia*, 55(10): 2565–2582. doi:10.1007/s00125-012-2644-8.
- Czech, M. P. (2017) Insulin action and resistance in obesity and type 2 diabetes. *Nat Med*, 23(7): 804–814. doi:10.1038/nm.4350.
- D'Alessandris, C., Andreozzi, F., Federici, M., Cardellini, M., Brunetti, A., Ranalli, M., & Sesti, G. (2004) Increased O-glycosylation of insulin signaling proteins results in their impaired activation and enhanced susceptibility to apoptosis in pancreatic beta-cells. *FASEB J*, 18(9): 959–961. doi:10.1096/fj.03-0725fje.
- David, H. W., William, G., Stephen, H., Diane, C., Tom, L., Michael, B., Deborah, De., Gregg, W., Don, P., Cheryl, D., Don, L., & George, M. (1998) Rapid, automated assay for progesterone on the Abbott AxSYM™ analyzer, *Clinical Chemistry*, 44(1):86–91, <https://doi.org/10.1093/clinchem/44.1.86>.
- Deng, F., Zhang, L., Lyu, L., Lu, Z., Gao, D., Ma, X., Guo, Y., Wang, R., Gong, S., & Jiang, W. (2021) Increased levels of ferritin on admission predicts intensive care unit mortality in patients with COVID-19. *Med Clin*, 156(7): 324–331. doi: 10.1016/j.medcli.2020.11.030.
- de Beer, T. A. P., Berka, K., Thornton, J. M., & Laskowski, R. A. (2014) PDB sum additions, *Nucl Acids Res*, 42(1): D292–D296.
- de Lemos, E. T., Oliveira, J., Pinheiro, J. P., & Reis, F. (2012) Regular physical exercise as a strategy to improve antioxidant and anti-inflammatory status: benefits in type 2 diabetes mellitus. *Oxid Med Cell Longev*, 741545:1–15 doi:10.1155/2012/741545.

- De Marinis, Y. Z., Salehi, A., Ward, C. E., Zhang, Q., Abdulkader, F., Bengtsson, M., & Amisten, S. (2010) GLP-1 inhibits, and adrenaline stimulates glucagon release by differential modulation of N-and L-type Ca²⁺ channel-dependent exocytosis. *Cell Metabolism*, 11(6):543–553.
- De Meyts, P. & Whittaker, J. (2002) Structural biology of insulin and IGF1 receptors: implications for drug design. *Nat Rev Drug Disc*, 1:769–783.
- De Meyts P. (2000) The Insulin Receptor and Its Signal Transduction Network. [Updated 2016 Apr 27]. In: Feingold, K. R., Anawalt, B., & Boyce, A editors. *Endotext* [Internet]. South Dartmouth(MA):MDText.com,Inc.;Availablefrom:<https://www.ncbi.nlm.nih.gov/books/NBK378978/>
- Deshpande, N., Address, K. J., Bluhm, W. F., Merino-Ott, J. C., Townsend-Merino, W., & Zhang, Q. (2005). The RCSB Protein Data Bank: a redesigned query system and relational database based on the mmCIF schema. *Nucl Acids Res*, 33(1):D233–D237. <https://doi.org/10.1093/nar/gki057>.
- Di Pino, A., & DeFronzo, R. A. (2019) Insulin Resistance and Atherosclerosis: Implications for Insulin-Sensitizing Agents. *Endocr Rev*, 40(6):1447–1467. doi:10.1210/er.2018-00141.
- Ding, W., Cheng, H., Yan, Y., Zhao, X., Chen, F., Huang, G., & Mi, J. (2016) 10-Year Trends in Serum Lipid Levels and Dyslipidemia Among Children and Adolescents From Several Schools in Beijing, China. *J Epidemiol*, 26(12):637–645. doi:10.2188/jea.JE2014025.
- Diz-Chaves, Y., Herrera-Pérez S., González-Matías L. C., Lamas J. A., & Mallo, F. (2020) Glucagon-Like Peptide-1 (GLP-1) in the Integration of Neural and Endocrine Responses to Stress. *Nutrients*, 12(11):3304. doi: 10.3390/nu12113304.
- Dhama, K., Khan, S., Tiwari, R., Sircar, S., Bhat, S., Malik, Y. S., & Rodriguez-Morales, A. J. (2020) Coronavirus Disease 2019-COVID-19. *Clin Microbiol Rev*, 33(4):e00028–20. doi:10.1128/CMR.00028-20.

- Eichler, T. E., Becknell, B., Easterling, R. S., Ingraham, S. E., Cohen, D. M., Schwaderer, A. L., & Spencer, J. D. (2016) Insulin and the phosphatidylinositol 3-kinase signaling pathway regulate Ribonuclease 7 expression in the human urinary tract. *Kidney Int*, 90(3):568–579. doi:10.1016/j.kint.2016.04.025.
- Fan, N., Peng, L., Xia, Z., Zhang, L., Song, Z., Wang, Y., & Peng, Y. (2019) Triglycerides to high-density lipoprotein cholesterol ratio as a surrogate for nonalcoholic fatty liver disease: a cross-sectional study. *Lipids Health Dis*, 18(1):39. doi:10.1186/s12944-019-0986-7.
- Fan, Z., Chen, L., Li, J., Cheng, X., Yang, J., Tian, C., Zhang, Y., Huang, S., Liu, Z., & Cheng, J. (2020) Clinical features of COVID-19- related liver functional abnormality. *Clin Gastroenterol Hepatol*, 18(7):1561–1566. doi:10.1016/j.cgh.2020.04.002.
- Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem*, 18(6):499–502.
- Fruman, D. A., Chiu, H., Hopkins, B. D., Bagrodia, S., Cantley, L. C., & Abraham, R. T. (2017) The PI3K Pathway in Human Disease. *Cell*, 170(4):605–635. doi:10.1016/j.cell.2017.07.029.
- Gao, J., Pan, X., Li, G., Chatterjee, E., & Xiao, J. (2021) Physical Exercise Protects Against Endothelial Dysfunction in Cardiovascular and Metabolic Diseases. *J Cardiovasc Transl Res*, 1–17: doi:10.1007/s12265-021-10171-3.
- Ghosh, A., Gao, L., Thakur, A., Siu, P. M., & Lai, C. W. K. (2017) Role of free fatty acids in endothelial dysfunction. *J Biomed Sci*, 24(1):50. doi:10.1186/s12929-017-0357-5.
- Grobler, C., Maphumulo S. C., Grobbelaar L. M., Bredenkamp J. C., Laubscher G. J., & Lourens P. J. (2020) Covid-19: The Rollercoaster of Fibrin (Ogen), D-Dimer, Von Willebrand Factor, P-Selectin and Their Interactions with Endothelial Cells, Platelets and Erythrocytes. *Int J Mol Sci*, 21(14):5168. doi: 10.3390/ijms21145168.

- Guptha, S., Gupta, R., Deedwania, P., Bhansali, A., Maheshwari, A., Gupta, A., & Sharma, K. K. (2014) Cholesterol lipoproteins and prevalence of dyslipidemias in urban Asian Indians: a cross sectional study. *Indian Heart J*, 66(3):280–288. doi:10.1016/j.ihj.2014.03.005.
- Guan, W., Ni, Z., Hu, Y., Liang, W., Ou, C., & He, J. (2020) Clinical Characteristics of Coronavirus Disease 2019 in China. *N Eng J Med*, 382(18):17081720. doi:10.1056/NEJMoa2002032.
- Haeusler, R. A., McGraw, T. E., & Accili, D. (2018) Biochemical and cellular properties of insulin receptor signalling. *Nat Rev Mol Cell Biol*, 19(1):31–44. doi:10.1038/nrm.2017.89.
- Hakim, A., Hasan, M. M., Hasan, M., Lokman, M. S., Azim, K. F., Raihan, T., Chowdhury, P. A., & Azad, A. K. (2021) Major Insights in Dynamics of Host Response to SARS-CoV-2: *Front. Microbiol*, 12(637554):1–28. doi103389/fmicb.2021637554.
- Henning, R. J. (2021) Obesity and obesity-induced inflammatory disease contribute to atherosclerosis: a review of the pathophysiology and treatment of obesity. *Am J Cardiovasc Dis*, 11(4):504–529.
- Henry, R.R. (1998). Type 2 diabetes care: the role of insulin-sensitizing agents and practical implications for cardiovascular disease prevention. *American Journal Medicine*, 105(1A):20S–26S. doi: 10.1016/s0002-9343(98)00207-1. PMID: 9707264.
- Herman, W. H., & Zimmet, P. (2012) Type 2 diabetes: an epidemic requiring global attention and urgent action. *Diabetes Care*, 35(5):943–944. doi:10.2337/dc12-0298.
- Heilbronn, L. K., Civitarese, A. E., Bogacka, I., Smith, S. R., Hulver, M., & Ravussin, E. (2005) Glucose tolerance and skeletal muscle gene expression in response to alternate day fasting. *Obesity research*, 13(3):574–81.
- Hill, M., Parizek, A., Simjak, P., Koucky, M., Anderlova, K., Krejci, H., & Kancheva, R. (2021) Steroids, steroid associated substances and gestational diabetes mellitus. *Physiol Res*, 70(Suppl4): S617–S634. doi:10.33549/physiolres.934794.

- Hill, N. R., Levy, J. C., & Matthews, D. R. (2013) Expansion of the homeostasis model assessment of β -cell function and insulin resistance to enable clinical trial outcome modeling through the interactive adjustment of physiology and treatment effects: iHOMA2. *Diabetes care*, 36(8): 2324–2330.
- Hudda, M. T., Donin, A. S., Owen, C. G., Rudnicka, A. R., Sattar, N., Cook, D. G., & Nightingale, C. M. (2019) Exploring the use of adjusted body mass index thresholds based on equivalent insulin resistance for defining overweight and obesity in UK South Asian children. *Int J Obes (Lond)*, 43(7):1440–1443. doi:10.1038/s41366-018-0279-7.
- Huang, I., Pranata, R., Lim, M. A., Oehadian, A., & Alisjahbana, B. (2020) C-reactive protein, procalcitonin, D-dimer, and ferritin in severe coronavirus disease-2019: a meta-analysis. *Ther Adv Respir Dis*, 14:1753466620937175. doi:10.1177/1753466620937175.
- Hubbard, M. J. & Cohen, P. (1993) On target with a new mechanism for the regulation of protein phosphorylation. *Trends Biochem Sci*, 18(5):172–7.
- IDF, G. (2011) ISPAD guideline for diabetes in childhood and adolescence. *International Diabetes Federation*, 131. *Ind J Pedi*, 81:165–169.
- Inokuchi, J., Kabayama, K., Nagafuku, M., & Sato, T. (2008) Regulation of insulin receptor function in microdomains. *Tanpakushitsu Kakusan Koso*, 53(12 Suppl):1552–1557.
- Imierska, M., Kurianiuk, A., & Błachnio-Zabielska, A. (2020) The Influence of Physical Activity on the Bioactive Lipids Metabolism in Obesity-Induced Muscle Insulin Resistance. *Biomolecules*, 10(12):1665. <https://doi.org/10.3390/biom10121665>.
- Janus, A., Szahidewicz-Krupska, E., Mazur, G., & Doroszko, A. (2016) Insulin Resistance and Endothelial Dysfunction Constitute a Common Therapeutic Target in Cardiometabolic Disorders. *Mediators Inflamm*, 3634948. doi:10.1155/2016/3634948.

- Jezek, P., Jaburek, M., & Plecita-Hlavata, L. (2019) Contribution of Oxidative Stress and Impaired Biogenesis of Pancreatic beta-Cells to Type 2 Diabetes. *Antioxid Redox Signal*, 31(10):722–751. doi:10.1089/ars.2018.7656.
- Jiang, S., Du, L., & Shi, Z. (2020a) An emerging coronavirus causing pneumonia outbreak in Wuhan, China: calling for developing therapeutic and prophylactic strategies. *Emerging microbes & infections*, 9(1):275–277 doi:10.1080/22221751.2020.1723441.
- Jiang, S., Xia, S., & Ying, T. (2020b) A novel coronavirus (2019-nCoV) causing pneumonia-associated respiratory syndrome. *Cellular & molecular immunology*, 17(5):554. doi:10.1038/s41423-020-0372-4.
- Jin, A., Yan, B., Hua, W., Feng, D., Xu, B., Liang, L., & Guo C. (2020) Clinical characteristics of patients diagnosed with COVID-19 in Beijing. *Biosaf Health*, 2(2):104–111 doi:10.1016/j.bsheat.2020.05.003.
- Jolliffe, I. T. (2002) *Principal component analysis*. second ed. New York: Springer-Verlag New York, Inc. url: <https://link.springer.com/book/10.1007/b98835>
- Joseph, L., Wasir, J. S., Misra, A., Vikram, N. K., Goel, K., & Pandey, R. M. (2011) Appropriate values of adiposity and lean body mass indices to detect cardiovascular risk factors in Asian Indians. *Diabetes technology & therapeutics*, 13(9):899-906.
- Kahn, C. R., & Folli, F. (1993) Molecular determinants of insulin action. *Horm Res*, 39(3):93–101. doi:10.1159/000182793.
- Kahn, B. B., & Flier, J. S. (2000) Obesity and insulin resistance. *J Clin Investig*, 106(4):473–81.
- Kalupahana, N. S., Massiera, F., Quignard-Boulangé, A., Ailhaud, G., Voy, B. H., & Wasserman, D. H. (2012) Overproduction of angiotensinogen from adipose tissue induces adipose inflammation, glucose intolerance, and insulin resistance. *Obesity*, 20(1): 48–56. doi:10.1038/oby.2011.299.
- Kawamoto, R., Tabara, Y., Kohara, K., Miki, T., Kusunoki, T., & Takayama, S. (2011) Relationships between lipid profiles and metabolic syndrome, insulin resistance and

- serum high molecular adiponectin in Japanese community-dwelling adults. *Lipids Health Dis*, 10 (79):1–7 doi:10.1186/1476-511X-10-79.
- Kasuga, M. (2019) Structure and function of the insulin receptor-a personal perspective. *Proc Jpn Acad Ser B Phys Biol Sci*, 95(10):581–589. doi:10.2183/pjab.95.039.
- Kasuga, M., Zick, Y., Blithe, D. L., Crettaz, M., & Kahn, C. R. (1982) Insulin stimulates tyrosine phosphorylation of the insulin receptor in a cell-free system. *Nature*, 298(5875):667–669. doi:10.1038/298667a0.
- Kaur, R., Kaur, M., & Singh, J. (2018) Endothelial dysfunction and platelet hyperactivity in type 2 diabetes mellitus: molecular insights and therapeutic strategies. *Cardiovasc Diabetol*, 17(1):121. doi:10.1186/s12933-018-0763-3.
- Kazemina, M., Salari, N., & Mohammadi, M. (2020) Prevalence of Cardiovascular Disease in Patients with Type 2 Diabetes Mellitus in Iran: A Systematic Review and Meta-Analysis. *J Diabetes Res*, 3069867. doi:10.1155/2020/3069867.
- Kashyap, S. R., Roman, L. J., Lamont, J., Masters, B. S., Bajaj, M., & Suraamornkul, S. (2005) Insulin resistance is associated with impaired nitric oxide synthase activity in skeletal muscle of type 2 diabetic subjects. *J Clin Endocrinol Metab*, 90(2):1100–1105.
- Kadowaki, T., Kadowaki, H., Rechler, M. M., Serrano-Rios, M., Roth, J., Gorden, P., & Taylor, S. I. (1990) Five mutant alleles of the insulin receptor gene in patients with genetic forms of insulin resistance. *J Clin Invest*, 86(1):254–64. doi:10.1172/JCI114693.
- Kharroubi, A. T., & Darwish, H. M. (2015) Diabetes mellitus: the epidemic of the century. *World J Diabetes*, 6(6):850.
- Kim, D., Lee, J.-Y., Yang, J.-S., Kim, J. W., Kim, V. N., & Chang, H. (2020) The Architecture of SARS-CoV-2 Transcriptome. *Cell*, 181(4):914–921.e10. doi.org/10.1016/j.cell.2020.04.011.
- Klein, S. L. & Flanagan, K. L. (2016) Sex differences in immune responses. *Nat Rev Immunol*, 16(10):626–638. doi:10.1038/nri.2016.90.

- Knutson, M. D. (2017) Iron transport proteins: Gateways of cellular and systemic iron homeostasis. *J Biol Chem*, 292(31):12735-12743 doi:10.1074/jbc.R117.786632.
- Kovats, S. (2015) Estrogen receptors regulate innate immune cells and signaling pathways. *Cell Immunol*. 294 (2):63–69.
- Koozi, H., Lengquist, M., & Frigyesi, A. (2020) C-reactive protein as a prognostic factor in intensive care admissions for sepsis: A Swedish multicenter study. *J Crit Care*, 56:73–79.
- Kumar, A., Arora, A., Sharma, P., Anikhindi, S. A., Bansal, N., & Singla, V. (2020) Is diabetes mellitus associated with mortality and severity of COVID-19? A meta-analysis. *Diabetes Metab Syndr*, 14(4):535–545. doi:10.1016/j.dsx.2020.04.044.
- Krishnamoorthy, Y., Rajaa, S., Murali, S., Rehman, T., Sahoo, J., & Kar, S. S. (2020) Prevalence of metabolic syndrome among adult population in India: A systematic review and meta-analysis. *PLoS One*, 15(10): e0240971. doi:10.1371/journal.pone.0240971.
- Lalueza, A., Ayuso, B., Arrieta, E., Trujillo, H., Folgueira, D., & Cueto, C; INFLUDOC group. (2020) Elevation of serum ferritin levels for predicting a poor outcome in hospitalized patients with influenza infection. *Clin Microbiol Infect*, 26(11):1557.e1559–1557.e1515.
- Lair, B., Laurens, C., Van Den Bosch, B., & Moro, C. (2020) Novel Insights and Mechanisms of Lipotoxicity-Driven Insulin Resistance. *Int J Mol Sci*, 21(17):6358. doi:10.3390/ijms21176358
- Laws, A., & Reaven, G. M. (1992) Evidence for an independent relationship between insulin resistance and fasting plasma HDL-cholesterol, triglyceride and insulin concentrations. *J Intern Med*, 231(1):25-30.
- Lee, B. J., Nam, J., & Kim, J. Y. (2016) Predictors of metabolic abnormalities in phenotypes that combined anthropometric indices and triglycerides. *BMC Complement Altern Med*, 16 (59): doi:10.1186/s12906-016-1024-1.

- Lee, J., & Pilch, P. F. (1994) The insulin receptor: structure, function, and signaling. *Am J Physiol*, 266(2 Pt 1): C319–34.
- Lee, Y., Chakraborty, S., Meininger, C. J., & Muthuchamy, M. (2018) Insulin resistance disrupts cell integrity, mitochondrial function, and inflammatory signaling in lymphatic endothelium. *Microcirculation*, 25(7): e12492. doi:10.1111/micc.12492.
- Li, H., Wang, Y., Ji, M., Pei, F., Zhao, Q., & Zhou, Y., Hong, Y. (2020) Transmission Routes Analysis of SARS-CoV-2: A Systematic Review and Case Report. *Front Cell and Dev Biol*, 8(618): doi:10.3389/fcell.2020.00618.
- Lim, S., Bae, J. H., Kwon, H. S., & Nauck, M. A. (2021) COVID-19 and diabetes mellitus: from pathophysiology to clinical management. *Nat Rev Endocrinol*, 17(1):11–30. doi:10.1038/s41574-020-00435-4.
- Linkins, L. A. & Takach Lapner, S. (2017) Review of D-dimer testing: Good, Bad, and Ugly. *Int J Lab Hematol*, 39(Suppl1):98–103. doi:10.1111/ijlh.12665.
- Lippi, G., Lavie, C. J., & Sanchis-Gomar, F. (2020) Cardiac troponin I in patients with coronavirus disease 2019 (COVID-19): Evidence from a meta-analysis. *Prog Cardiovasc Dis*, 63(3):390–391 doi:10.1016/j.pcad.2020.03.001.
- Liu, F., Li, L., Xu, M., Wu, J., Luo, D., Zhu, Y., Li, B., Song, X., & Zhou, X. (2020) Prognostic value of interleukin-6, C-reactive protein, and procalcitonin in patients with COVID-19. *J Clin Virol*, 127:104370. doi: 10.1016/j.jcv.2020.104370.
- Li, C. C., Young, P. E., Maloney, C. A., Eaton, S. A., Cowley, M. J., & Buckland, M. E. (2013) Maternal obesity and diabetes induces latent metabolic defects and widespread epigenetic changes in isogenic mice. *Epigenetics*, 8(6):602–611. doi:10.4161/epi.24656.
- Lin, S. Y., Li, W. C., Yang, T. A., Chen, Y. C., Yu, W., & Huang, H. Y. (2021) Optimal Threshold of Homeostasis Model Assessment of Insulin Resistance to Identify Metabolic Syndrome in a Chinese Population Aged 45 Years or Younger. *Front Endocrinol (Lausanne)*, 12:746747. doi:10.3389/fendo.2021.746747.

- Lim, S., Bae, J. H., Kwon, H. S., & Nauck, M. A. (2021) COVID-19 and diabetes mellitus: from pathophysiology to clinical management. *Nat Rev Endocrinol*, 17(1):11–30. doi:10.1038/s41574-020-00435-4.
- Longo, N., Wang, Y., & Smith, S. A. (2002) Genotype-phenotype correlation in inherited severe insulin resistance, *Human Mol Gen*, 11(12):1465–1475.
- Lopes-Verila, M. E., Stone, P., & Ellis, S. (1977) Cholesterol determination in high-density lipoproteins separation by three different methods. *Clinical Chemistry*, 23(5):882–884.
- Lumeng, C. N., Deyoung, S. M., Bodzin, J. L., & Saltiel, A. R. (2007) Increased inflammatory properties of adipose tissue macrophages recruited during diet-induced obesity. *Diabetes*, 56(1):16–23. doi:10.2337/db06-1076.
- Manna, P., & Jain, S. K. (2015) Phosphatidylinositol-3,4,5-triphosphate and cellular signaling: implications for obesity and diabetes. *Cell Physiol Biochem*, 35(4):1253–1275. doi:10.1159/000373949.
- Marucci, A., Miscio, G., Padovano, L., Boonyasrisawat, W., Florez, J. C., Doria, A., & Paola, R. D. (2009) The role of HSP70 on ENPP1 expression and insulin-receptor activation. *J Mol Med (Berl)*, 87(2):139–144. doi:10.1007/s00109-008-0429-9.
- Mather, K., Anderson, T. J., & Verma, S. (2001). Insulin action in the vasculature: physiology and pathophysiology. *J Vasc Res*, 38(5):415–422. doi:10.1159/000051074.
- Maude, H., Sanchez-Cabanillas, C., & Cebola, I. (2021) Epigenetics of Hepatic Insulin Resistance. *Front Endocrinol (Lausanne)*, 12:681356. doi:10.3389/fendo.2021.681356.
- Mao, R., Qiu, Y., He, J. S., Tan, J. Y., Li, X. H., & Liang, J., Shen, J. Manifestations and prognosis of gastrointestinal and liver involvement in patients with COVID-19: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol*, 5(7):667–678. doi: 10.1016/S2468-1253(20)30126-6.

- Mallah, S. I., Ghorab, O. K., Al-Salmi, S., Abdellatif, O. S., Tharmaratnam, T., Iskandar, M. A., & Al-Qahtani, M. (2021) COVID-19: breaking down a global health crisis. *Ann Clin Microbiol Antimicrob*, 20(1):35. doi:10.1186/s12941-021-00438-7.
- McDonald, J. H. (2008) *Handbook of biological statistics*. Baltimore: Sparky House Publishing. url: <https://www.biostathandbook.com>
- Mehta, P., McAuley, D. F., Brown, M., Sanchez, E., Tattersall, R. S., & Manson, J. J. (2020) COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet*, 395(10229):1033–1034.
- Mei, H., Li, L., Jiang, F., Simino, J., Griswold, M., Mosley, T., & Liu, S. (2016) *snpGeneSets: An R Package for Genome-Wide Study Annotation*. *G3 (Bethesda)*, 6(12):4087–4095. doi:10.1534/g3.116.034694.
- Meiquer (2006) *Insulin Glucose Metabolism*, online resource, accessed 22/1/2007 <http://en.wikipedia.org/wiki/Image:Insulin_glucose_metabolism.jpg. [O’Sullivan, T.A. (2008). *The Relationship Between Glycemic Intake and Insulin Resistance in Older Women*. PhD thesis. School of Public Health, Faculty of Health and the Institute of Health and Biomedical Innovation, Queensland University of Technology. 1–226. https://www.researchgate.net/publication/27476581_The_relationship_between_glycemic_intake_and_insulin_resistance_in_older_women (Retrieved on January 1, 2023 at 10:00 am).]
- Miao, B., Skidan, I., Yang, J., Lugovskoy, A., Reibarkh, M., Long, K., & Degterev, A. (2010) Small molecule inhibition of phosphatidylinositol-3,4,5-triphosphate (PIP3) binding to pleckstrin homology domains. *Proc Natl Acad Sci U S A*, 107(46):20126–20131. doi:10.1073/pnas.1004522107.
- Miao, Z., Alvarez, M., Ko, A., Bhagat, Y., Rahmani, E., & Jew, B. (2020) The causal effect of obesity on prediabetes and insulin resistance reveals the important role of adipose tissue in insulin resistance. *PLoS Genet*, 16(9): e1009018. doi: 10.1371/journal.pgen.1009018.

- Moller, D. E., Yokota, A., White, M. F., Pazianos, A. G., & Flier, J. S. (1990) A naturally occurring mutation of insulin receptor alanine 1134 impairs tyrosine kinase function and is associated with dominantly inherited insulin resistance. *J Biol Chem*, 265(25):14979–14985. doi.org/10.1016/S0021-9258(18)77212-8.
- Muniyappa, R., Chen, H., Montagnani, M., Sherman, A., & Quon, M. J. (2020) Endothelial dysfunction due to selective insulin resistance in vascular endothelium: insights from mechanistic modeling. *Am J Physiol Endocrinol Metab*, 319(3):E629–E646. doi:10.1152/ajpendo.00247.2020.
- Musicki, B., Kramer, M. F., Becker, R. E., & Burnett, A. L. (2005) Inactivation of phosphorylated endothelial nitric oxide synthase (Ser-1177) by O-GlcNAc in diabetes-associated erectile dysfunction. *Proc Natl Acad Sci U S A*, 102(33):11870–11875. doi:10.1073/pnas.0502488102.
- Musambil, M., & Siddiqui, K. (2019) Genetics and genomics studies in type 2 diabetes: a brief review of the current scenario in the Arab region. *Diabetes Metab Syndr*, 13(2):1629–1632.
- Nagao, H., Kashine, S., Nishizawa, H., Okada, T., Kimura, T., Hirata, A., & Shimomura, I. (2013) Vascular complications and changes in body mass index in Japanese type 2 diabetic patients with abdominal obesity. *Cardiovasc Diabetol*, 12(1):1–9 doi:10.1186/1475-2840-12-88.
- Napoleao, A., Fernandes, L., Miranda, C., & Marum, A. P. (2021) Effects of Calorie Restriction on Health Span and Insulin Resistance: Classic Calorie Restriction Diet vs. Ketosis-Inducing Diet. *Nutrients*, 13(4):1302. doi:10.3390/nu13041302.
- Neff, A. M., Yu, J., Taylor, R. N., Bagchi, I. C., & Bagchi, M. K. (2020) Insulin Signaling Via Progesterone-Regulated Insulin Receptor Substrate 2 is Critical for Human Uterine Decidualization. *Endocrinology*, 161(1):1–20 bqz021. doi:10.1210/endo/bqz021.
- Oo, Y. H., Karam, J. G., & Resta, C. A. (2013) Extreme insulin resistance in a patient with diabetes ketoacidosis and acute myocardial infarction. *Case Rep Endocrinol*, 520904:1–7 doi:10.1155/2013/520904.

- O'Beirne, S. L., Salit, J., Rodriguez-Flores, J. L., Staudt, M. R., Abi Khalil, C., Fakhro, K. A., & Zirie, M. (2018) Exome sequencing-based identification of novel type 2 diabetes risk allele loci in the Qatari population. *PloS One*, 13(9):199–207.
- Ormazabal, V., Nair, S., Elfeky, O., Aguayo, C., Salomon, C., & Zuniga, F. A. (2018) Association between insulin resistance and the development of cardiovascular disease. *Cardiovasc Diabetol*, 17(1):122. doi:10.1186/s12933-018-0762-4.
- Olver, T. D., Grunewald, Z. I., Ghiarone, T., Restaino, R. M., Sales, A. R. K., & Park, L. K. (2019) Persistent insulin signaling coupled with restricted PI3K activation causes insulin-induced vasoconstriction. *Am J Physiol Heart Circ Physiol*, 317(5): H1166–H1172.
- O'Sullivan, T.A. (2008) The Relationship Between Glycemic Intake and Insulin Resistance in Older Women. PhD Thesis. School of Public Health, Faculty of Health and the Institute of Health and Biomedical Innovation, Queensland University of Technology, Australia. P.11.
- Paliogiannis, P., Zinellu, A., Scano, V., Mulas, G., De Riu, G., & Pascale, R. M. (2020) Laboratory test alterations in patients with COVID-19 and non COVID-19 interstitial pneumonia: a preliminary report. *J Infect Dev Countr*, 14(7):685–690.
- Pal R, & Bhadada S. K. (2020) COVID-19 and diabetes mellitus: An unholy interaction of two pandemics. *Diabetes Metab Syndr*, 14(4):513–517 doi:10.1016/j.dsx.2020.04.049.
- Petersen, M. C., & Shulman, G. I. (2018) Mechanisms of Insulin Action and Insulin Resistance. *Physiol Rev*, 98(4):2133–2223. doi:10.1152/physrev.00063.2017.
- Phan, L. T., Nguyen, T. V., Luong, Q. C., Nguyen, T. V., Nguyen, H. T., & Le, H. Q. (2020) Importation and Human-to-Human Transmission of a Novel Coronavirus in Vietnam. *N Engl J Med*, 382(9):872–874 doi:10.1056/NEJMc2001272.
- Pinti, M. V., Fink, G. K., Hathaway, Q. A., Durr, A. J., Kunovac, A., & Hollander, J. M. (2019) Mitochondrial dysfunction in type 2 diabetes mellitus: an organ-based analysis. *Am J Physiol Endocrinol Metab*, 316(2): E268–E285. doi:10.1152/ajpendo.00314.2018.

- Pinzi, L., & Rastelli, G. (2019) Molecular Docking: Shifting Paradigms in Drug Discovery. *Int J Mol Sci*, 20(18):4331 doi:10.3390/ijms20184331.
- Png, M. E., Yoong, J., Phan, T. P., & Wee, H. L. (2016) Current and future economic burden of diabetes among working-age adults in Asia: conservative estimates for Singapore from 2010-2050. *BMC Public Health*, 16:153. doi:10.1186/s12889-016-2827-1.
- Potenza, M. A., Addabbo, F., & Montagnani, M. (2009) Vascular actions of insulin with implications for endothelial dysfunction. *Am J Physiol Endocrinol Metab*, 297(3): E568–577. doi:10.1152/ajpendo.00297.2009.
- Purushottam, L., Adusumalli, S. R., Singh, U., Unnikrishnan, V. B., Rawale, D. G., & Gujrati, M. (2019) Single-site glycine-specific labeling of proteins. *Nat Commun*, 10(1):2539.
- Rahman, M.S., Hossain, K.S., Das, S.; Kundu, S., Adegoke, E.O., Rahman, M.A., Hannan, M.A., Uddin, M.J., & Pang, M.-G. (2021) Role of Insulin in Health and Disease: An Update. *Int. J. Mol. Sci*, 22(12):6403. doi.org/10.3390/ijms22126403 .
- Rabi, F. A., Al Zoubi, M. S., Kasasbeh, G. A., Salameh, D. M., & Al-Nasser, A. D. (2020) SARS-CoV-2 and Coronavirus Disease 2019: What We Know So Far. *Pathogens* 9(3): 231. doi:10.3390/pathogens9030231.
- Ramasamy, S., & Subbian, S. (2021) Critical Determinants of Cytokine Storm and Type I Interferon Response in COVID-19 Pathogenesis. *Clin Microbiol Rev*, 34(3): e00299-20. doi:10.1128/CMR.00299-20.
- Rabiee, A., Kruger, M., Ardenkjaer-Larsen, J., Kahn, C. R., & Emanuelli, B. (2018) Distinct signalling properties of insulin receptor substrate (IRS)-1 and IRS-2 in mediating insulin/IGF-1 action. *Cell Signal*, 47:1–15. doi:10.1016/j.cellsig.2018.03.003
- Radhakrishna, P., Vinod, K. V., Sujiv, A., & Swaminathan, R. P. (2018) Comparison of Hemoglobin A1c with Fasting and 2-h Plasma Glucose Tests for Diagnosis of Diabetes and Prediabetes among High-risk South Indians. *Indian J Endocrinol Metab*, 22(1):50–56. doi:10.4103/ijem.IJEM_254_17

- Radenkovic, S. P., Kocic, R. D., Pesic, M. M., Dimic, D. N., Golubovic, M. D., & Radojkovic, D. B. (2011) The hypertriglyceridemic waist phenotype and metabolic syndrome by differing criteria in type 2 diabetic patients and their relation to lipids and blood glucose control. *Endokrynologia Polska*, 62(4):316–23.
- Rajan, M. R., Fagerholm, S., Jonsson, C., Kjolhede, P., Turkina, M. V., & Stralfors, P. (2013) Phosphorylation of IRS1 at serine 307 in response to insulin in human adipocytes is not likely to be catalyzed by p70 ribosomal S6 kinase. *PLoS One*, 8(4): e59725. doi:10.1371/journal.pone.0059725.
- Reaven, G. (2012) Insulin resistance and coronary heart disease in nondiabetic individuals. *Arterioscler Thromb Vasc Biol*, 32(8):1754–9.
- Ross, R. M., Wadley, G. D., Clark, M. G., Rattigan, S., & McConell, G. K. (2007) Local nitric oxide synthase inhibition reduces skeletal muscle glucose uptake but not capillary blood flow during in situ muscle contraction in rats. *Diabetes*, 56(12):2885–2892. doi:10.2337/db07-0745.
- Ryan, M., Diekhans, M., Lien, S., Liu, Y., & Karchin, R. (2009) LS-SNP/PDB: annotated non-synonymous SNPs mapped to Protein Data Bank structures, *Bioinformatics*, 25(11): 1431–1432.
- Ryoo, S. M., Han, K. S., Ahn, S., Shin, T. G., Hwang, S. Y., & Chung, S. P; Korean Shock Society (KoSS) Investigators. (2019) The usefulness of C-reactive protein and procalcitonin to predict prognosis in septic shock patients: A multicenter prospective registry-based observational study. *Sci Rep*, 9(1):6579.
- Salber, P. R., Bestermann, W., Schwartz, S., & Marchetti, A. (2008) Loss of confidence in diabetes management. *Managed Care (Langhorne, Pa.)*, 17(10):38–46.
- Sanyaolu, A., Okorie, C., Marinkovic, A., Patidar, R., Younis, K., & Desai, P. (2020) Comorbidity and its Impact on Patients with COVID-19. *SN Compr Clin Med*, 2(8):1069–10761. doi:10.1007/s42399-020-00363-4
- Sakurai, Y., Kubota, N., Yamauchi, T., & Kadowaki, T. (2021) Role of Insulin Resistance in MAFLD. *Int J Mol Sci*, 22(8):4156 doi:10.3390/ijms22084156.

- Samuel, V. T., & Shulman, G. I. (2012) Mechanisms for insulin resistance: common threads and missing links. *Cell*, 148(5):852–871. doi:10.1016/j.cell.2012.02.017.
- Sarmiento, B. E., Santos Menezes, L. F., & Schwartz, E. F. (2019) Insulin Release Mechanism Modulated by Toxins Isolated from Animal Venoms: From Basic Research to Drug Development Prospects. *Molecules*, 24(10):1846. doi:10.3390/molecules24101846.
- Scapin, G., Dandey, V. P., Zhang, Z., Prosise, W., Hruza, A., Kelly, T., & Carragher, B. (2018) Structure of the insulin receptor-insulin complex by single-particle cryo-EM analysis. *Nature*, 556(7699):122–125. doi:10.1038/nature26153.
- Semiz, S., Dujic, T., & Causevic, A. (2013) Pharmacogenetics and personalized treatment of type 2 diabetes. *Biochem Med (Zagreb)*, 23(2):154–171. doi:10.11613/bm.2013.020.
- Sethuraman, N., Jeremiah, S. S., & Ryo, A. (2020) Interpreting Diagnostic Tests for SARS-CoV-2. *Jama*, 323(22):2249–2251doi:10.1001/jama.2020.8259.
- Shah, P., Doshi, R., Chenna, A., Owens, R., Cobb, A., Ivey, H., Newton, S., & McCarley, K. (2020) Prognostic value of elevated cardiac troponin i in hospitalized Covid-19 patients. *Am J Cardiol*, 135:150–153. doi: 10.1016/j.amjcard.2020.08.041.
- Simon, H. (2010) Mars vs. Venus: the gender gap in health. *Harvard Men’s Health Watch*. 14(6):1–5.
- Sproston, N. R., & Ashworth J. J. (2018) Role of C-reactive protein at sites of inflammation and infection. *Front Immunol*, 9:754. doi: 10.3389/fimmu.2018.00754.
- Smith, U. & B. B. Kahn (2016) Adipose tissue regulates insulin sensitivity: role of adipogenesis, de novo lipogenesis and novel lipids. *J Intern Med*, 280(5): 465–475.
- Softic, S., Gupta, M. K., Wang, G. X., Fujisaka, S., O'Neill, B. T., Rao, T. N., & Kahn, C. R. (2017) Divergent effects of glucose and fructose on hepatic lipogenesis and insulin signaling. *J Clin Invest*, 127(11):4059-4074. doi:10.1172/JCI94585.

- Sortica, D. A., Buffon, M. P., Souza, B. M., Nicoletto, B. B., Santer, A., Assmann, T. S., & Canani, L. H. (2015) Association between the ENPP1 K121Q polymorphism and risk of diabetic kidney disease: a systematic review and meta-analysis. *PLoS One*, 10(3): e0118416. doi:10.1371/journal.pone.0118416
- Son, N. E. (2019) Influence of ferritin levels and inflammatory markers on HbA1c in the Type 2 Diabetes mellitus patients. *Pak J Med Sci*, 35(4):1030–1035 doi:10.12669/pjms.35.4.1003.
- Sproston, N. R., & Ashworth, J. J. (2018) Role of C-reactive protein at sites of inflammation and infection. *Front Immunol*, 9:754. doi: 10.3389/fimmu.2018.00754.
- Sramek, J., Nemcova-Furstova, V., & Kovar, J. (2021) Molecular Mechanisms of Apoptosis Induction and Its Regulation by Fatty Acids in Pancreatic beta-Cells. *Int J Mol Sci*, 22(8): doi:10.3390/ijms22084285.
- Steinthorsdottir, V., Thorleifsson, G., & Reynisdottir, I. (2007) A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. *Nat Genet*, 39:770–775. doi:10.1038/ng2043.
- Stratford, S., De Wald, D. B., & Summers, S. A. (2001) Ceramide dissociates 3'-phosphoinositide production from pleckstrin homology domain translocation. *Biochem J*, 354(Pt 2): 359–368. doi:10.1042/0264-6021:3540359.
- Taguchi, A., & White, M. F. (2008) Insulin-like signaling, nutrient homeostasis, and life span. *Annu Rev Physiol*, 70:191-212. doi:10.1146/annurev.physiol.70.113006.100533.
- Tallapragada, D. S., Karpe, P. A., & Tikoo, K. (2015) Long-lasting partnership between insulin resistance and endothelial dysfunction: role of metabolic memory. *Br J Pharmacol*, 172(16):4012–4023. doi:10.1111/bph.13145.
- Tan, S. X., Fisher-Wellman, K. H., Fazakerley, D. J., Ng, Y., Pant, H., Li, J., & James, D. E. (2015) Selective insulin resistance in adipocytes. *J Biol Chem*, 290(18):11337–11348. doi:10.1074/jbc.M114.623686.

- Tan, J., Liu, S., Zhuang, L., Chen, L., Dong, M., Zhang, J., & Xin, Y. (2020) Transmission and clinical characteristics of asymptomatic patients with SARS-CoV-2 infection. *Future Virology*, 10.2217/fvl-2020-0087. doi:10.2217/fvl-2020-0087.
- Tang, N., Li, D., Wang, X., & Sun, Z. (2020) Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J thromb Haemost*, 18(4):844-847 doi:10.1111/jth.14768.
- Thachil, J., Tang, N., Gando, S., Falanga, A., Cattaneo, M., Levi, M., Clark, C., & Iba, T. (2020) ISTH interim guidance on recognition and management of coagulopathy in COVID-19. *J Thromb Haemost*, 18(5):1023–1026.
- Tomic, D., Shaw, J.E. & Magliano, D.J. (2022) The burden and risks of emerging complications of diabetes mellitus. *Nat Rev Endocrinol*, 18(9):525–539. doi: 10.1038/s41574-022-00690-7.
- Trouwborst, I., Bowser, S. M., Goossens, G. H., & Blaak, E. E. (2018) Ectopic Fat Accumulation in Distinct Insulin Resistant Phenotypes; Targets for Personalized Nutritional Interventions. *Front Nutr*, 5:77. doi:10.3389/fnut.2018.00077.
- Triolo, T. M., Fouts, A., Pyle, L., Yu, L., Gottlieb, P. A., Steck, A. K; Type 1 Diabetes Trial Net Study Group. (2019) Identical and Nonidentical Twins: Risk and Factors Involved in Development of Islet Autoimmunity and Type 1 Diabetes. *Diabetes Care*, 42(2):192–199.
- Usui, I., Imamura, T., Huang, J., Satoh, H., & Olefsky, J. M..(2003) Cdc42 is a Rho GTPase family member that can mediate insulin signaling to glucose transport in 3T3-L1 adipocytes. *J Biol Chem*, 278(16):13765–74.
- Van den Oever, I. A., Raterman, H. G., Nurmohamed, M. T., & Simsek, S. (2010) Endothelial dysfunction, inflammation, and apoptosis in diabetes mellitus. *Mediators Inflamm*, 792393. doi:10.1155/2010/792393
- Vargas-Vargas M, & Cortés-Rojo C. (2020) Ferritin levels and COVID-19. *Rev Panam Salud Publica*, 44:e72–e72.

- Veghari, G., Sedaghat, M., Maghsodlo, S., Banihashem, S., Moharloei, P., & Angizeh, A. (2013) The correlation between educational levels and central obesity in the north of Iran: an epidemiologic study. *ARYA Atherosclerosis*, 9(4):217–22.
- Vivanti, A. J., Vauloup-Fellous, C., Prevot, S., Zupan, V., Suffee, C., & Do Cao, J. (2020) Transplacental transmission of SARS-CoV-2 infection. *Nature Communications*, 11(1):3572 doi:10.1038/s41467-020-17436-6.
- Wu, Y., Li, H., & Loos, R. J. F. (2008) Common variants in CDKAL1, CDKN2A/B, IGF2BP2, SLC30A8, and HHEX/IDE genes are associated with type 2 diabetes and impaired fasting glucose in a Chinese Han population. *Diabetes*, 57(10):2834–2842. doi:10.2337/db08-0047.
- Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., & Zhang, J. (2020a) Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA*, 323(11):1061–9. doi:10.1001/jama.2020.1585.
- Wang, T., Wang, J., Hu, X., Huang, X. J., & Chen, G. X. (2020) Current understanding of glucose transporter 4 expression and functional mechanisms. *World J Biol Chem*, 11(3): 76–98. doi:10.4331/wjbc.v11.i3.76.
- Wang, W., Tang, J., & Wei, F. (2020b) Updated understanding of the outbreak of 2019 novel coronavirus (2019-nCoV) in Wuhan, China. *J Med Virol*, 92(4):441-447 doi:10.1002/jmv.25689.
- White, M. F., & Kahn, C. R. (2021) Insulin action at a molecular level - 100 years of progress. *Mol Metab*, 52:101304. doi:10.1016/j.molmet.2021.101304.
- WHO. (2016) Diabetes Country Profiles. In: World Health Organization Geneva, Switzerland.
- WHO. (2017) WHO Clinical consortium on healthy ageing: Topic focus: Frailty and intrinsic capacity: Report of Consortium Meeting, 1–2 December 2016 in Geneva, Switzerland.
- WHO. (2020a) Geneva: 2020. Coronavirus disease 2019 (COVID-19) situation report 51. <https://www.who.int/docs/default-source/coronaviruse/situation->

- reports/20200311-sitrep-51-covid-19.pdf?sfvrsn=1ba62e57_10 Available at: [Accessed 07 August 2020] [Google Scholar].
- WHO. (2020b) Geneva: 2020. Coronavirus disease (COVID-19) pandemic. <https://www.who.int/emergencies/diseases/novel-coronavirus-2019> Available at: [Accessed 07 August 2020]
- WHO. (2020) Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: interim guidance, <https://apps.who.int/iris/handle/10665/331329>. Accessed: 6 May 2020. Worldometer Coronavirus Cases. 2020. 1-22.
- Xu, J., Zhao, S., Teng, T., Abdalla, A. E., Zhu, W., Xie, L., Wang, Y., & Guo, X. (2020) Systematic Comparison of Two Animal-to-Human Transmitted Human Coronaviruses: SARS-CoV-2 and SARS-CoV. *Viruses*, 12(2):244. doi:10.3390/v12020244.
- Xu, Y., & Qiao, J. (2022) Association of Insulin Resistance and Elevated Androgen Levels with Polycystic Ovarian Syndrome (PCOS): A Review of Literature. *J Healthc Eng*, 9240569:1–13 doi:10.1155/2022/9240569.
- Yadav, R., Hama, S., Liu, Y., Siahmansur, T., Schofeld, J., Syed, A. A., France, M., Pemberton, P., Adam, S., & Ho, J. H. (2017) Effect of Roux-en-Y bariatric surgery on lipoproteins, insulin resistance, and systemic and vascular inflammation in obesity and diabetes. *Front Immunol*, 8:1512.
- Yanai, H., & Yoshida, H. (2019) Beneficial Effects of Adiponectin on Glucose and Lipid Metabolism and Atherosclerotic Progression: Mechanisms and Perspectives. *Int J Mol Sci*, 20(5):1190. doi:10.3390/ijms20051190.
- Yaribeygi, H., Sathyapalan, T., Atkin, S. L., & Sahebkar, A. (2020) Molecular Mechanisms Linking Oxidative Stress and Diabetes Mellitus. *Oxid Med Cell Longev*, 8609213:1–13 doi:10.1155/2020/8609213.
- Youngren, J. F. (2007) Regulation of insulin receptor function. *Cell Mol Life Sci*, 64(7–8):873–891. doi:10.1007/s00018-007-6359-9.

- Yu, B., Li, X., Chen, J., Ouyang, M., Zhang, H., & Zhao, X. (2020) Evaluation of variation in D-dimer levels among COVID-19 and bacterial pneumonia: a retrospective analysis. *J Thromb Thrombolysis*, 50:548–557. doi:10.1007/s11239-020-02171-y.
- Zabidi, N. A., Ishak, N. A., Hamid, M., Ashari, S. E., & Latif, M. A. (2021) Inhibitory evaluation of *Curculigo latifolia* on alpha-glucosidase, DPP (IV) and in vitro studies in antidiabetic with molecular docking relevance to type 2 diabetes mellitus. *J Enzyme Inhib Med Chem*, 36(1):109–121. doi:10.1080/14756366.2020.1844680
- Zheng, Z., Peng, F., Xu, B., Zhao, J., Liu, H., Peng, J., Li, Q., Jiang, C., Zhou, Y., & Liu, S. (2020) Risk factors of critical & mortal COVID-19 cases: A systematic literature review and meta-analysis. *J Infect*, 81(2): e16–e25.
- Zheng, C., Hu, M., & Gao, F. (2017) Diabetes and pulmonary tuberculosis: a global overview with special focus on the situation in Asian countries with high TB-DM burden. *Glob Health Action*, 10(1):1–11. doi:10.1080/16549716.2016.1264702.
- Zhang, J. J., Dong, X., Cao, Y. Y., Yuan, Y. D., Yang, Y. B., & Yan, Y. Q. (2020) Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy*, 75(7):1730–1741 doi:10.1111/all.14238.
- Zheng, Z., Peng, F., Xu, B., Zhao, J., Liu, H., & Peng, J. (2020) Risk factors of critical & mortal COVID-19 cases: A systematic literature review and meta-analysis. *J Infect* 81(2): e16–e25 doi:10.1016/j.jinf.2020.04.021.
- Zhou, B., She, J., Wang, Y., & Ma, X. (2020). Utility of ferritin, procalcitonin, and C-reactive protein in severe patients with 2019 novel coronavirus disease. *Research Square*,1–10. DOI: 10.21203/rs.3.rs-18079/v1.
- Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., & Liu, Z., Xiang, J. (2020a) Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*, 395(10229):1054–1062 doi:10.1016/S0140-6736(20)30566-3.
- Zhou, P., Yang, X. L., Wang, X. G., Fan, G., Liu, Y., & Liu, Z. (2020b) A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, 579(7798):270–273 doi:10.1038/s41586-020-2012-7.

- Zhou, J., Massey, S., Story, D., & Li, L. (2018) Metformin: An Old Drug with New Applications. *Int J Mol Sci*, 19(10):2863. doi: 10.3390/ijms19102863.
- Zu, Z. Y., Jiang, M. D., Xu, P. P., Chen, W., Ni, Q. Q., Lu, G. M., & Zhang, L. J. (2020) Coronavirus Disease 2019 (COVID-19): A Perspective from China. *Radiology*, 296(2): E15–E25. doi:10.1148/radiol.202020.