SINGLE NUCLEOTIDE POLYMORPHISM IN ADIPOKINE GENES AND ITS CORRELATION WITH PLASMA LEVEL OF ADIPOKINES IN INDIVIDUALS WITH PREDIABETES

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

Clinical Biochemistry

By

Bineesh C P

Registration Number: 41800728

Supervised By

Dr. Pranav Kumar Prabhakar (16113)

Professor

Department of Medical Laboratory Sciences

Lovely Professional University, Phagwara, Punjab

Co-Supervised by

Dr. Vipin Viswanath

Professor and Head,

Department of Transplant Immunology and

Molecular Diagnostic Laboratory,

Aster MIMS Calicut, Kerala

LOVELY PROFESSIONAL UNIVERSITY, PUNJAB
2024

DECLARATION

I hereby declare that the thesis entitled, **"Single Nucleotide Polymorphism in Adipokine Genes and its Correlation with Plasma Level of Adipokines in Individuals with Prediabetes"** submitted for Ph.D. in Clinical Biochemistry Degree to Department of Medical Laboratory Sciences, School of Allied Medical Sciences, Lovely Professional University is entirely original work and all ideas and references have been duly acknowledged. The research work has not formed the basis for the award of any other degree.

Signature

Name of the scholar:

BINEESHCP

Registration No. : 41800728
Department of Medical Laboratory Sciences, School of Allied

Medical Sciences, Lovely Professional University,

Punjab, India

CERTIFICATE

This is to certify that **Mr. Bineesh C P** has completed the Ph.D. Biochemistry titled, **"Single Nucleotide Polymorphism in Adipokine Genes and its Correlation with Plasma Level of Adipokines in Individuals with Prediabetes"** under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigation and study. No part of this thesis hasever been submitted for any other degree or diploma. The thesis is fit for the partial fulfillment of the condition for the award of the degree of Ph.D. in Clinical Biochemistry.

Signature of Supervisor

Dr. Pranav Kumar Prabhakar

Professor

Department of Medical Laboratory Sciences,

Lovely Professional University, Phagwara, Punjab

ABSTRACT

India, which has the second-largest population in the world, may overtake China as the most populated country in the world by 2024.

Numerous studies have been conducted over the last few decades in an effort to determine the incidence of diabetes in India. In India, where above 74 million people have been analyzed with the condition, diabetes is quickly spreading like an epidemic. Estimates from the International Diabetes Federation indicate that 642 million people will have DM by 2040, up from an estimated 415 million in 2015. Type 2 Diabetes Mellitus (T2DM) is one of the primary causes of mortality, accounting for over 1.7 million deaths annually. T2DM is the one of the common forms of diabetes. Additionally, etiology is complicated and includes both hereditary and behavioral variables. The prevalence of diabetes is high and rising both globally and in developing countries like India, mostly as a result of increased rates of overweight/obesity and unhealthy lifestyles.

By 2030, 80 to 100 million people in India may have T2DM, according to estimates. Kerala, a federal state in India, is exceptional since its health metrics are on level with those of wealthy nations. T2DM, however, is now very noticeable throughout Kerala's entire community. Diabetes and atherosclerosis are mostly attributed to inflammation. A probable link between elevated levels of adiposity and an up regulation of cytokines in prediabetes, which results in vascular inflammation and is a causative factor for T2DM.

Historically, adipose tissue has been thought of as an organ for long-term energy storage. Adipose tissue dysfunction is the major defect in obesity and may raise the risk of T2DM, fatty liver, and cardiovascular disease, among other health issues. Adipokines are a group of substances that are released by adipose tissue. These are the hormones and cytokines which consist of pro- and anti-inflammatory effects. It affects metabolic homeostasis, satiety, and reproduction.

There are few inflammatory markers in prediabetes, which is indicated by Glycated hemoglobin A1C (HbA1c) levels between 5.7 and 6.4% as well as impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). Leptin, which is produced in small amounts by the human obese (OB) gene, is found in human tissues such as the stomach, mammary epithelium, placenta, and heart. Numerous studies have also established the role of leptin therapy in hypoleptinemic patients' glucose and lipid metabolism.

Resistin, an adipokine, has been linked to obesity, insulin resistance, and diabetes.

Several single-nucleotide polymorphisms (SNPs) in adipokine genes, such as leptin (LEP), resistin (RETN), interleukin, and adiponectin (ADIPOQ), have not yet been studied.

Adiponectin is a member of the adipocytokines family of peptide hormones secreted by fat cells. Adiponectin, the most abundant adipocytokine, increases insulin sensitivity in its target tissues, which may explain why obese people have lower levels of adiponectin. Adipokines such as adiponectin, leptin, and resistin play an important role in body weight regulation, particularly through fat metabolism.

There has been limited research on the link between genetic variations of the adipokine gene and prediabetes, a condition that puts people at greater risk of developing T2DM. However, the associations between these genetic polymorphisms of adipokine genes ad its association with the concentration of plasma adipokines among individuals with prediabetes was not yet studies well. The present study hypothesizes that the adiposity or their related complications in prediabetes cause significant changes in adipokines level hence designed to investigate the significance of adipokine levels and insulin resistance and estimate the association between these for the prediction of prediabetes.

Aim of the present study was to identify the association of adipokine single nucleotide gene polymorphism (SNP) and the plasma levels of adipokines in prediabetes individuals in the north Kerala population. A case-control study was conducted in Aster Mims Hospital, Calicut, Kerala with 150 prediabetes subjects (40 obese and 110 non-obese) and 150 controls; age group from 30 to 50 years of both sexes. Age and sex-matched healthy control subjects without prediabetes and any family history of diabetes recruited. A detailed assessment that includes, demographic data (age, sex, height, weight, etc.), family history and medical history was recorded. Oral glucose tolerance test (OGTT) was carried among the study subjects. Blood samples were collected in EDTA tubes for the extraction of DNA. The study subjects were classified into groups according to the result of HbA1c and OGTT as, to normal control group (N=150) and pre-diabetes group (N=150). According to BMI, those individuals with pre-diabetes were again classified into two groups as, obese (N=40) and non-obese (N=110).

The single nucleotide polymorphism of adiponectin (ADIPOQ-rs266729), leptin (LEP2548- rs7799039), and resistin (RETN420- rs1862513) were assessed by Real-Time PCR. The genotyping result for each SNP marker was verified using DNA sequencing. The plasma adipokines were also estimated by ELISA. Statistical analysis

was performed using IBM SPSS version 21.0 software (Chicago USA). Numerical variables were represented using mean and standard deviation. ANOVA was analyzed and which is followed by multiple linear regression. Moreover, Pearson correlation was also analyzed. Allelic and genotype frequencies were presented with 95% confidence interval (C.I).

The findings of the current study were, serum adiponectin levels decreased significantly in obese prediabetes when compared to the rest. Serum leptin levels elevated significantly among obese prediabetes than the others. Adiponectin leptin ratio decreased significantly in obese prediabetes. Correlation between adipokine levels and insulin resistance are statistically insignificant.

There was no significant difference between adiponectin level, leptin level, resistin level and gene polymorphism of ADIPOQ11377(C>G) ($\chi2$=1.549, p=0.46), LEP2548 (G>A) ($\chi2$= 0.797, p=0.67). RETN420 (C>G) ($\chi 2$ = 1.549, p=0.08) in control group.

There was a significant association between adiponectin level, leptin level and gene polymorphism of ADIPOQ, LEP2548 in non-obese prediabetic group. There was a significant association between adiponectin, leptin level and gene polymorphism of ADIPOQ 11377(C>G) ($\chi2$=12.46, p=0.002), LEP2548 (G>A) ($\chi2$=6.97, p=0.03) in obese prediabetic group, and not significant association between resistin level and RETN 420 polymorphism ($\chi2$=1, p=0.61). The association of all genotype and allele polymorphism of adiponectin and leptin were found highly statistically significant, whereas none of the resistin were showed any significant association.

From these findings and results it was concluded that, the obtained results suggest that adipokine profile and insulin resistance may useful markers in the identification of individuals with risk of developing metabolic syndrome as well as predictors of prediabetes. Findings the current study indicated that plasma adiponectin level and ADIPOQrs266729C>G polymorphism and plasma leptin level and LEP2548-rs7799039 G>A polymorphism may contribute to genetic risk of prediabetes and provide preliminary data useful in genetic screening for prediabetes among the Kerala population.

ACKNOWLEDGEMENT

TABLE OF CONTENTS

List of Figures

List of Tables

LIST OF ABBREVIATIONS

ADA	American diabetic association
ADIPOQ	Adiponectin
ADP	Adenosine triphosphate
AMP	Adenosine Monophosphate
ANOVA	Analysis of Variance
AT	Adipose tissue
BMI	Body Mass Index
BMS	Bare Metal Stent
BP	Blood Pressure
BSA	Bovine Serum Albumin
BW	Buffer BW
CAD	Coronary artery disease
CDC	Centers for Disease Control and Prevention
CHD	Coronary Heart Disease
CI	Confidence Interval
CLIA	Chemical Luminescence Immunoassay
CRP	C-reactive protein
CVD	Cardiovascular Disease
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid
EC	Ethics Committee
ECLIA	Electrochemical Luminescence Immunoassay
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-Linked Immunoassay
FBG	Fasting blood glucose
FBS	Fasting Blood Sugar
FPG	Fasting Plasma Glucose
FRET	Förster Resonance Energy Transfer
GOD-POD	Glucose oxidase – Peroxidase
GWAS	Genome Wide Association Studies
HDL	High-Density Lipoprotein
HMW	High-Molecular-Weight
HOMA	Homeostasis Model Assessment
HPLC	High Performance Liquid Chromatography
HRP	Horseradish Peroxidase

ICMR	Indian Council of Medical Research
IDF	The International Diabetes Federation
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
IL	Interleukin
IMT	intima-media thickness
IR	Infrared radiation
LEP	Leptin
LEPR	Leptin Receptor
NAFLD	Non-alcoholic fatty liver disease
NGSP	National Glycohemoglobin Standardization Program
NGT	National Green Tribunal
OGTT	Oral Glucose Tolerance Tests
PAPS	Phosphoadenosine phosphosulfate
PBS	Phosphate-buffered saline
PCOS	Polycystic Ovary Syndrome
PCR	Polymerase Chain Reaction
PPARs	Peroxisome Proliferator-Activated Receptors
PPBS	Postprandial Glucose Test
RETN	Resistin
RNA	Ribonucleic Acid
SD	Standard Deviation
SNP	Single Nucleotide Polymorphism
SPSS	Statistical Package for the Social Sciences
TC	Total Cholesterol
TG	Triglyceride
TMB	3,3′,5,5′-Tetramethylbenzidine
TNF	Tumor Necrosis Factor
TZD	Thiazolidinedione
USA	United States of America
UV	Ultra violet
VCAM 1	Vascular Cell Adhesion Molecule 1
WC	Waist Circumference
WHO	World Health Organisation
WHR	Waist-Hip Circumference Ratio

CHAPTER-1

INTRODUCTION

1.1 PREDIABETES

Prediabetes represents a condition with an elevated risk of progressing to diabetes, defined as blood glucose concentrations higher than normal but lower than diabetes thresholds. Prediabetes criteria have evolved over time and differ depending on the institution of origin (Tabák *et al.,* 2012). Prediabetes is characterized by elevated blood glucose levels that exceed the normal range but fall below the diagnostic threshold for diabetes (Bansal *et al.,* 2015).

The diagnostic criteria for prediabetes include elevated levels of fasting plasma glucose, a glycated hemoglobin (HbA1c) value, or increased plasma glucose levels following an oral glucose tolerance test (World Health Organization (WHO, 2006)).

Prediabetes which is also referred to as intermediate hyperglycemia increases the risk for diabetes. Approximately 5% to 10% cases are converted to diabetes. The glycemic index in such cases is above what is said to be normal however slightly below the diabetic threshold (Tabák *et al*., 2012). Diabetes affected 77 million people in India in 2019, with the number expected to rise to more than 134 million by 2045 (Pradeepa *et al*., 2021). ICMR conducted a community screening that led to the finding that Northern India is less affected than its southern counterpart. (Anjana *et al.,* 2011).

The extended duration of the disease and the rising incidence of early-onset diabetes contribute to the emergence of diverse diabetic complications. However, comprehensive data on the prevalence of diabetic complications throughout India remains limited (Mohan et al., 2013). Prediabetes definitions hinge on assessments of fasting glucose levels, two-hour plasma glucose levels during an oral glucose tolerance test (OGTT), and/or levels of HbA1c (Lee et al., 2011), with the latter inclusion being a more recent addition to the definition. Nevertheless, there has been ongoing debate regarding its practical utility in clinical settings (Nowicka et al., 2011).

1.2 INCIDENCE AND PREVALENCE OF PRE-DIABETES

Diabetes and prediabetes are rapidly growing in India. The prevalence of prediabetes is increasing globally, with authorities estimating that even more than 470 million people will always have prediabetes by 2030 (Tabák *et al*., 2012). Prediabetes is a stage that precedes T2DM (WHO 2006). With 69.2 million T2DM patients, India ranks second only to China in the world (Sen *et al*., 2002). Kerala, India's federal state, is exceptional in that its health indicators are comparable to those of developed countries (Brown 2013). Diabetes is a chronic condition that has caused serious health crises worldwide and is ranked among top ten causes of fatality along with other chronic diseases like cardiovascular diseases (CVD), respiratory disease, and cancer. (Parayil 1996).

In prediabetes, insulin resistance is recompensed for by a higher serum insulin level. According to the American Diabetes Association, 70% of people with prediabetes will probably develop T2DM (Freeman and Pennings 2022).

ADA introduced a new hemoglobin A1C (HbA1C)-based description of prediabetes in 2010, along with the first suggestions for using HbA1C to diagnose diabetes (Sherwani *et al*., 2016).

1.3 ROLE OF SNPs IN PRE - DIABETES

The metabolic disruptions in individuals with insulin resistance are manifold and intricate, as noted by Mihai et al. (2014). Among the well-recognized adipokines, adiponectin and leptin stand out, and there seems to be a connection with clinical cardiovascular disease. However, attempts in prior studies to establish a quantitative relationship have produced conflicting outcomes, as indicated by Van de Voorde et al. (2013).

Significant alterations take place, particularly in adipose tissue, which is now recognized for its active hormonal production, referred to as adipokines (Yadav *et al*., 2013). Changes in adipokine secretion are commonly linked with decreased insulin activity Mihai *et al*., (2014). Adipose tissue is considered an endocrine organ. It is a complex structure that, in addition to fat storage, releases a number of bioactive polypeptides known as "adipokines" (Kershaw *et al*., 2004).

Adipose tissue serves as a crucial endocrine organ, releasing a diverse range of

factors referred to as adipocytokines or adipokines (Inadera 2008). A huge proportion of molecular epidemiological studies have looked at the relationship between various adipokine genetic polymorphisms and obesity. T2DM has a strong genetic link, according to genome wide association studies (GWAS), and many genes are responsible for developing T2DM and its complications (Prasad and Groop 2015).

Adipose tissue is a common endocrine organ that secretes a variety of adipokines (Coelho *et al.*, 2013). Numerous proteins produced by adipose tissue have been identified, potentially establishing a connection between insulin resistance, obesity, and the onset of diabetes. The ADIPOQ gene encodes adiponectin, a 244 amino acid protein. The ADIPOQ gene is located on the 3q27 chromosomal region, which is a susceptibility locus for T2DM (Al-Harithy *et al.*, 2012). A functional SNP, rs266729, in the ADIPOQ gene's promotor region causes (Avery *et al.*, 2011).

Despite the fact that the G allele of ADIPOQ rs266729 appears to be important in association with T2DM risk in various populations (Park *et al.*, 2011). The ADIPOQ rs266729 C>G polymorphism could make a contribution to the genetic risk of prediabetes as well as provide preliminary data useful in prediabetes genetic screening among this population (Eissa *et al.*, 2016). A reduction in plasma adiponectin levels is vigorously linked to the development of T2DM and obesity. In Jordan, serum adiponectin levels and SNPs in the ADIPOQ gene are linked to prediabetes (Engert *et al.*, 2002).

Adiponectin, the most abundant adipokine synthesized by adipocytes, is recognized for its impact on insulin sensitivity, with reduced levels observed in individuals with obesity (Yang and Chuang, 2006). The gene responsible for encoding adiponectin is the adiponectin C1Q and collagen domain-containing (ADIPOQ) gene, situated on chromosome 3q27 (De Luis *et al.*, 2020). Many polymorphisms in ADIPOQ have been linked to adiponectin levels, insulin resistance, and obesity in humans (Takahashi *et al.*, 2000). The ADIPOQ rs266729 polymorphism helps to regulate adiponectin promoter activity and, in turn, adiponectin levels (Gu 2009). ADIPOQ variants have been linked to adiponectin levels, a rise in BMI, decreased insulin sensitivity, and diabetic kidney disorder (Rasmussen-Torvik *et al.*, 2009).

ADIPOQ rs266729 C>G polymorphism may contribute to the genetic risk of prediabetes and provide preliminary data useful in the genetic screening for

prediabetes among this (Eissa., 2016). The ADIPOQ rs266729 polymorphism actively modulates the promoter activity of adiponectin, consequently influencing adiponectin levels (Gu., 2009).

Leptin is an adipokine hormone that is primarily secreted by adipocytes. The human obesity gene leptin has a 16KD protein with 164 amino acids (Jiang *et al*., 2016). The human leptin gene (LEP) is located on 7q31.3 and includes three exons and two introns spanning roughly 18kb (Li *et al*., 2015). Obesity, metabolic syndrome, and hypertension are all linked to elevated plasma leptin levels (Nogueiras *et al*., 2010). Leptin increases cell sensitivity to insulin, indicating that patients with prediabetes have resistance to leptin action, resulting in higher insulin levels (Bungau *et al*., 2020).

Leptin regulates body fat by repressing appetite, increasing energy expenditure, and regulating glucose homeostasis (Amitani *et al*., 2013). genotypic distribution G-2548 In a group of Saudis, a variant of the LEP gene is linked to higher levels of plasma leptin and glucose (Sabi *et al*., 2022). Polymorphisms in the LEP gene as a risk factor for diabetes mellitus (Prior *et al*., 2009). In animal models, mutations that deactivate the gene responsible for encoding LEP have been associated with obesity, and LEP was initially recognized as the obese gene (Campfield *et al*., 1995). Serum leptin levels in obese people were found to be higher in many cohorts (Murea *et al*., 2012).

Based on this there is a hypothesis that leptin resistance can be a feature of obesity. (Hijjawi *et al*., 2018). Obese patients show elevated levels of insulin as well as leptin and there is experimental evidence that insulin enhances the secretion of leptin by adipose cells.(Bungau *et al*., 2020). Elevated insulin levels, conversely, hinder the normal hypothalamic reaction to leptin, which is responsible for suppressing appetite; weight loss serves to enhance this response (Tiwari-Heckler *et al*., 2018).

Resistin, a cysteine-rich peptide with a molecular weight of 12.5kD, is secreted by adipocytes in rodents and by macrophages in humans (Rosenthal et al., 2017). The human resistin gene (RETN) is situated on chromosome 19p13.3, and several identified single nucleotide polymorphisms (SNPs) are associated with T2DM (Skurk et al., 2007). Elevated serum resistin levels have been observed in individuals with obesity, insulin resistance, and T2DM (Suriyaprom et al.,

2015). Various studies, involving diverse population groups, have linked SNPs at the ADIPOQ and RETN loci to an increased risk of T2DM and metabolic syndrome (MS), but the results are often conflicting (Menzaghi et al., 2007; Henneman et al., 2010).

In the Japanese population, the GG genotype of the RETN -420C>G promoter polymorphism (rs1862513) has been associated with elevated fasting plasma resistin levels and an increased risk of type 2 diabetes (Osawa et al., 2004). The relationship between insulin resistance and resistin levels has also been established (Silha et al., 2003).

1.4 NEED FOR THE STUDY

Prediabetes is a state of impaired glucose tolerance (IGT) and impaired fasting glycemia (IFG). Studies are showing the link between genetic variations of the adipokine gene and prediabetes, a condition that puts people at greater risk of developing T2DM Currently, there were no reports available regarding the association between genetic variants of adipokine and prediabetes. However, the association of adipokine gene polymorphism and prediabetes symptoms are not yet elucidated. Correlation between plasma adipokine level and gene polymorphism in the prediabetes stage was not fully established. There exist controversial results regarding adipokine secretion and insulin resistance associated with T2DM. Moreover, the association of adipokine gene polymorphism and adipokine levels among prediabetes subjects was still unknown. Hence, the present study was undertaken to determine the association of adiposity or their related complications in prediabetes cause significant changes in adipokines level.

1.5 RESEARCH GAP

[1]. Previously no studies have investigated the association between genetic variants of adipokine and prediabetes, group at higher risk of T2DM.

[2]. Present study hypothesis the adiposity or its related complication in prediabetes individuals or significant changes in adipokines level.

[3]. In this study, the aim is to evaluate the correlation of adipokine gene polymorphism and the plasma level of adipokines (adiponectin, leptin, and resistin) in individuals with prediabetes.

[4].   The present study explains, molecular changes in this adipokine in relation to the prediabetic Kerala population

1.6. AIM

The aim was to evaluate the correlation of adipokine gene polymorphism and the plasma level of adipokines (adiponectin, leptin, and resistin) in individuals with prediabetes. The present study explains the molecular changes in this adipokine in relation to prediabetic Kerala population.

1.7 ETHICAL CONSIDERATION

The study was started after getting the ethical clearance and permission was granted by the Institutional Ethics Committee "(reg no: EC/NEW/INST/2019/406 & ECR/301/Inst/KL/2013/RR-19, Dated 27/02/2020)."

CHAPTER-2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The present study Single nucleotide polymorphism in adipokine genes and its correlation with plasma level of adipokines in individuals with prediabetes has been reviewed with the following headings:

2.1 INTRODUCTION

"Impaired fasting glucose (IFG)" is defined as *"Fasting plasma glucose (FPG)"* levels between under 100-120 mg/dl. People having this condition are more prone to developing diabetes (Balion *et al.,* 2007). People with IFG can have more advanced glycemic homeostasis disturbances than those with IGT; for example, among individuals of Caucasian descent, the concurrence of abnormalities in those with impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) can be as minimal as 25%. Those who exhibit both IFG and IGT tend to experience more advanced disruptions in glycemic homeostasis (Tabák *et al*., 2012).

According to current estimates, the majority of people with prediabetes (up to 70%) have DM (Nathan *et al*., 2007).

Every year, 5% to 10% of people with prediabetes develop diabetes (Forouhi *et al*., 2007). A strong and consistent link exists between HbA1c levels and the risk of developing diabetes (Zhang *et al*., 2010). If the HbA1c level was equal to or exceeded 6%, the likelihood of developing diabetes was twentyfold higher compared to when it was below 5% (Heianza *et al*., 2011). If current trends continue, by 2050, one in every three adults will have diabetes (Chouhan *et al*., 2016).

2.2 GLOBAL PREVALENCE OF PREDIABETES

The prevalence of IGT is estimated to be 7.5% based on a study by the International Diabetes Federation (IDF) conducted in 2019 in both the genders (Echouffo-Tcheugui and Selvin 2021). The latter projection pertains to approximately 374 million adults aged 18-99 years, with nearly half (48.1%) below the age of 50 and approximately one-third (28.3%) falling between the ages of 20 and 39 (Saeedi et al., 2019). The majority of individuals with prediabetes (72.2%) reside

in low- and middle-income countries (LMICs). Notably, North America and the Caribbean exhibit the highest prevalence of impaired glucose tolerance (IGT) at 13.8%, while Europe reports the lowest prevalence at 5.1% (Saito *et al*., 2011).

The 2019 International Diabetes Federation (IDF) estimates, lacking data on impaired fasting glucose (IFG) or glycated hemoglobin (HbA1c), potentially underestimate the prevalence of prediabetes compared to estimates incorporating all glycemic measures (Shen et al., 2016). Nevertheless, prediabetes prevalence remains unreported for numerous countries, and global figures rely on statistical extrapolations and considerable assumptions, introducing substantial uncertainty (Wang et al., 2013). Notably, even within the currently acknowledged normal range, an elevated fasting plasma glucose (FPG) level heightens the risk of developing diabetes (Shaw *et al*., 2000).

It is expected to reach 642 million people by 2040 (Khetan and Rajagopalan 2018). Several longitudinal studies have linked prediabetes (IGT and IFG) to an increased risk of cardiovascular events, with IGT being a slightly stronger risk predictor (Coutinho *et al*., 1999).

2.3 INCIDENCE AND PREVALENCE

There is a rapid worldwide increase in prevalence of T2DM in the last few decades (Zheng *et al*., 2018). It has affected 451 million adults in the age of 18- 99 years worldwide and there is an expected rise to 693 million by 2025. (Cho *et al*., 2018). Pre-diabetes prevalence obviously varies with the age range of the population studied, with older people having a higher prevalence (Beulens *et al*., 2019).

Europe alone is estimated to be having 58 million affected adults and this is expected to rise by 66.7 million in the next 20 years (Kyrou *et al*., 2020). Most of the diabetic cases go undiagnosed and thus remain untreated and thereby are at a higher risk of developing type 2 diabetes mellitus. In Europe alone, there are 22 million undiagnosed cases. (Zheng *et al*., 2018).

Diabetes prevalence has been reported to be increasing in both developed and developing countries (Danaei *et al*., 2011). When the data from the only five studies in middle-aged participants were combined, the overall overlap prevalence of pre-diabetes was 27% (Barry *et al*., 2017).

HbA1c as a T2DM criterion has sparked some debate, primarily because it results in a higher prevalence of pre-diabetes (Shahim *et al*., 2017). It is unclear whether people who meet these criteria are at an increased risk of developing T2DM or its cardiovascular complications (Yudkin and Montori 2014).

2.4 EPIDEMIOLOGY

The significance of intervening in adults with prediabetes is underscored by the outcomes of diabetes prevention trials (Knowler et al., 2022). These phenotypes are characterized by different levels of insulin resistance and beta-cell dysfunction, with impaired glucose tolerance (IGT) showcasing nearly maximal insulin resistance and an 80% reduction in beta-cell function (Lorenzo et al., 2016). Glycemic levels are experiencing rapid escalation in both developed and developing nations (Danaei *et al*., 2011).

Repeat testing is recommended every three years for those with normal findings, and every year for prediabetics (Abbasi *et al*., 2012). Various risk scores for T2DM are currently available, and a recent systematic review indicates that they exhibit a fair degree of comparability (Moebus et al., 2009). T2DM has emerged as a prominent global public health concern, with recent statistical analyses uncovering new epidemiological characteristics associated with the condition (Wu et al., 2014). Prediabetes can be identified through different criteria such as impaired glucose tolerance (IGT), impaired fasting glucose (IFG), and HbA1c levels (Mann et al., 2010). Utilizing a single measurement for diagnosing prediabetes may lead to some false-positive results (Ramachandran *et al*., 2016).

Among the ten most populous countries with the highest diabetic populations, seven are categorized as low- or middle-income nations, with India and China reporting prevalence rates of 9.7% each (Diamond et al., 2011). Advancing age is identified as a risk factor for type 2 diabetes (T2DM), and there is a growing concern regarding the escalating rates of childhood obesity in children, teenagers, and adolescents contributing to the risk (Yang et al., 2010). However, the lack of compelling high-quality evidence raises questions about the efficacy of universal screening in enhancing the prognosis of diabetes (Khetan *et al*., 2018).

T2DM is a high-risk condition with serious implications for disability,

including so-called glycemic disutility (Moebus *et al*., 2009). Patients with prediabetes may benefit from the inclusion of patients who are at high risk for future cardiovascular complications (Cusi *et al*., 2016). Glycemic markers like glycated albumin and fructosamine have the potential to detect prediabetes. (Juraschek *et al*., 2015).

Increases in glycemia have resulted in an increase in the prevalence of prediabetes (Danaei *et al*., 2011). In 2005-08, approximately 35% of US adults over the age of 20 and 50% of those over the age of 65 had prediabetes, as defined by FPG or HbA1c concentrations (Katikireddi *et al*., 2011). Insulin hypersecretion is required to compensate for peripheral insulin resistance and to preserve euglycemia (Kendall *et al*., 2009).

The prevalence of IFG and IGT varies by ethnic group, and both disorders are more common in people over the age of 40 (Cowie *et al*., 2006). Restoring normoglycemia during pre-diabetes or early T2DM is an effective deterrent to future progression (Kramer *et al*., 2013). When normoglycemia was reached, there was a 56% decrease in T2DM among patients who did not develop diabetes while participating in the study (Khetan and Rajagopalan 2018).

2.5 ETIOLOGY OF PRE-DIABETES

The pathogenesis of T2DM is influenced by various risk factors, encompassing demographic characteristics, metabolic syndrome, and specific unhealthy lifestyle behaviors (Khetan & Rajagopalan, 2018). Numerous studies have identified that several of the risk factors associated with diabetes are already prevalent in individuals with prediabetes (Qin and Xu, 2016). Diabetes is closely linked to various risk factors, including a familial history of diabetes, increased age, obesity, hypertension, and physical inactivity (Wang et al., 2017).

2.5.1 PROGRESSION TO DIABETES

The conversion rates of individuals from prediabetes to diabetes exhibit variability based on population characteristics and the criteria employed to define prediabetes (Forouhi et al., 2007). Diabetes was observed to have an annual incidence rate of 4%-6% for isolated impaired glucose tolerance (IGT), 6%-9% for isolated impaired fasting glucose (IFG), and 15%-19% for both IGT and IFG (Nathan et al.,

2007). The annual prevalence rates of progression from prediabetes to diabetes were found to be similar (Gerstein *et al.*, 2007).

Diabetes occurred at a rate of 7% in the HbA1c 5.7%-6.4% group and 9% in the IFG group (Heianza *et al.*, 2011). The results of operations of diabetes among participants in the control group with IGT was found to be more than 90% over a 20-year period, as determined by repeated OGTT (Li *et al.*, 2008).

Age, gender, ethnicity, fasting blood sugar, systolic blood pressure, HDL cholesterol, body mass index, and diabetes history are all easily accessible variables for diabetes risk scoring (Bansal 2015).

2.5.2 INSULIN AND PREDIABATES

T2DM is an intricate metabolic disorder distinguished by compromised insulin action and reduced insulin secretion (Kasuga et al., 2006). The pathophysiology is elucidated as insulin resistance induced by factors such as age or obesity, succeeded by a decline in the compensatory response of pancreatic β-cells (Gujral *et al.*, 2016).

T2DM arises from a gradual decline in insulin secretion, often accompanied by varying degrees of insulin resistance (DeFronzo *et al.*, 2011). Increased fasting insulin concentrations in prediabetic subjects indicate an increased state of insulin resistance (Abdul-Ghani *et al.*, 2006). T2DM is linked to cognitive dysfunction, spanning from mild cognitive impairment to dementia. (Willmann *et al.*, 2020). The longer you have diabetes, the more likely you are to develop mild cognitive impairment or dementia (Cheng *et al.*, 2012). In the prediabetic state, cognitive decline appears to begin well before the manifestation of overt diabetes (Yaffe *et al.*, 2006).

The primary factor leading to the transition from prediabetes to T2DM is the swift progression of insulin resistance, ultimately leading to the breakdown of a compensatory surge in insulin (Liu *et al.*, 2014). There is an ethnic difference in the rate of progression to T2DM, with those of South Asian and African origin having a higher conversion rate than those of European origin (Morimoto *et al.*, 2013). Insulin resistance can be found in a variety of tissues, including skeletal muscle, liver, and adipocytes (Groop *et al.*, 1989).

Diabetes prevalence has increased rapidly in India, coinciding with concurrent

economic, epidemiological, and nutritional transitions (Misra *et al.*, 2011). The pathogenetic link between impaired glycemic control and cognitive decline is still being investigated (Ekblad *et al.*, 2017). The major pathomechanisms in the development of type 2 diabetes mellitus are decreased insulin secretion and peripheral insulin resistance (Okereke *et al.*, 2010). Asian Indians have distinct biological characteristics, such as impaired pancreatic insulin production (Mohan *et al.*, 2013).

2.5.3 AGE

Most countries' life expectancy is increasing, and the global life expectancy is rising (Cho *et al.*, 2018). Given recent advancements in the survival of diabetic patients, it is not surprising that older age is now acknowledged as a significant risk factor for type 2 diabetes (T2DM) (Lee and Halter, 2017). The Search for Diabetes in Youth study, a landmark investigation, indicates that T2DM emerging at a young age tends to be more aggressive in nature (Al-Saeed et al., 2016).

There is a likelihood that increased exposure to hyperglycemia over a lifetime is associated with heightened risks of complications (Huo et al., 2018). Individuals diagnosed with T2DM before the age of 40 exhibit the highest excess risk for all-cause mortality, while those diagnosed after the age of 80 show no excess mortality (Sattar et al., 2019). A younger age of T2DM onset is likely linked to an elevated risk of all-cause mortality, underscoring the importance of efforts to prevent or delay the onset of T2DM (Zhuo et al., 2014).

The higher the lifetime excess medical costs associated with diabetes, the earlier in life diabetes develops (Ang 2020). Diabetes affected approximately 4.0% of people aged 18-44 years (CDC 2014). The age group 65-74 years had the highest prevalence of diabetes (Hirani and Ali 2006). T2DM prevalence was more than twice as high in the older generation as in the younger (Suastika *et al.*, 2011).

Patients with prediabetes are more likely to develop diabetes as well as cardiovascular disease (Kurihara *et al.*, 2013). Diabetes was linked to an increased risk of all-cause mortality of more than 10% (Huang *et al.*, 2014). Prediabetes-related free fatty acids and insulin resistance activate molecular mechanisms that alter the function and structure of blood vessels (Ferrannini 2014).

In comparison to individuals without diabetes, those who developed type 1

diabetes mellitus (T1DM) before the age of ten exhibited a threefold increase in mortality (Rawshani et al., 2018). The likelihood of developing T2DM increases with advancing age (Cho et al., 2018). Additionally, age has been demonstrated as an independent predictor of decreased daily physical activity levels (Davies et al., 2019). The prevalence of T2DM is projected to escalate from 122.8 million in 2017 to 253.4 million by the year 2045 (Harrison et al., 2003).

The age groups 60-74 (19%) and 75-79 (22%), respectively, had the highest diabetes prevalence (Colberg *et al*., 2016). The pathophysiological reason for this is that as we age, our bodies become less sensitive to insulin (Khan *et al*., 2019). Diabetes appears to be three times more prevalent in high-income countries among people aged 65 to 69 (Cho *et al*., 2018). In Europe, the general population aged 50-99 years is expected to increase to 53.6% by 2045 (Kyrou *et al*., 2020).

Prediabetes is a hyperglycemic intermediate state with glycemic parameters above normal but below the diabetes threshold (Neamah *et al*., 2016).

2.5.4 LIFESTYLE FACTORS

Obesity is the leading risk factor for T2DM (Cullmann *et al*., 2012). 90% of diabetic patients establish T2DM, which is primarily caused by excess body weight (Chen *et al*., 2012). Those who have a modifiable risk factor for insulin resistance, which leads to the development of prediabetes (Belkina and Denis 2010). T2DM patients are far more common (36%-60%) than the general population (Schobe *et al*., 2011).

Addressing obesity through enhanced physical activity and dietary modifications can effectively target modifiable risk factors associated with prediabetes and diabetes (Knowler et al., 2022; Bansal 2015).

The connection between cardiovascular risk and blood glucose levels extends progressively below the threshold for prediabetes (Buysschaert and Bergman, 2011). The association between glucose levels and the risk of developing coronary heart disease (CHD) remains continuous and graded across the spectrum of non-diabetic glucose concentrations, irrespective of conventional risk factors (Hoogwerf et al., 2022).

2.5.5 NEPHROPATHY AND KIDNEY DISEASE

A link between prediabetes and an increased risk of chronic kidney disease and early nephropathy (Planting *et al*., 2010). Rather than the effect of prediabetes, this group has a higher incidence of diabetes or the presence of other factors associated with both hyperglycemia and nephropathy (Thomas *et al*., 2011).

2.5.6 OBESITY

Diabesity is a new term that has been coined to better describe the twin epidemics of T2DM and obesity (Zheng *et al*., 2018). Obesity is now acknowledged as the most important modifiable risk factor for prediabetes and T2DM (American Diabetes Association 2010). An independent positive association between increasing BMI and the risk of T2DM has also been clearly documented (Kyrou *et al*., 2018).

The prediabetic population already has impaired glucose regulation (Mijajlović *et al*., 2017). Obesity affects 90% of diabetic patients. In 2010, Holman *et al*., expressed that, Obesity raises the levels of adipocytes, cytokines (interleukin-1 (IL-1) and IL-6, as well as tumor necrosis factor-alpha (TNF- α) in the body (Echouffo-Tcheugui and Selvin 2021).

The cumulative incidence of diabetes among prediabetic subjects in the non-intervention group was greater than 90% (Li *et al*., 2008). Visceral and ectopic fat accumulations are now recognized as major risk factors for T2DM, directly related to hyperinsulinemia and insulin resistance (Kyrou *et al*., 2018). Independent of BMI, a positive relationship between central/visceral obesity and T2DM risk has been documented (Klein *et al*., 2007).

An increase in the concentration of these components activates a signaling pathway that represents adipose tissue inflammation (Kim *et al*., 2008). In insulin-resistant obese patients, mRNA expression of IL-1 and IL-6 was higher than in non-insulin-resistant obese patients (Purkayastha *et al*., 2011). T2DM prevalence increases with waist circumference, and the latter is a significant independent predictor of T2DM (Lewitt *et al*., 2010).

2.5.7 NEUROPATHIES

Diabetes has been linked to decreased parasympathetic modulation of the heart (Tesfay *et al*., 2010). Aside from glycemic control, smoking was associated with an

increased risk of neuropathy (OR = 1.68) (Clair *et al*., 2015). A few studies have looked into the link between smoking and the risk of diabetic neuropathy (Tesfaye *et al*., 2005).

Non-invasive evaluation of neural impairment in IGT subjects revealed significantly more abnormalities (Putz *et al*., 2009). There is mounting evidence that prediabetics have a higher incidence of idiopathic polyneuropathy and small fibre neuropathy (Nebuchennykh *et al*., 2008). During prediabetes, the small unmyelinated nerve fibres that carry pain, temperature, and regulate autonomic function are involved (Sumner *et al*., 2003). There were 1550 new cases of diabetic neuropathy observed; the risk of neuropathy among smokers was not significantly increased (Tesfaye *et al*., 2005).

2.5.8   PHYSICAL ACTIVITY

Increased sedentary time and lower levels of physical activity contribute to a high risk of prediabetes (Zheng *et al*., 2018). The global age-standardized prevalence of insufficient physical activity was 27.5%, with women and high-income countries having even higher rates (Weisman *et al*., 2018). Sedentism has been identified as a major contributor to the development of prediabetes (Wild *et al*., 2006).

Higher BMI and older age were both associated with higher rates of increase in sedentary time and decrease in physical activity in both men and women (Kyrou *et al*., 2020). Alarming trends are an important component of T2DM prevention, especially in individuals at high risk of T2DM (Colberg *et al*., 2016). People who watched more TV had a 112% higher pool for T2DM than people who watched less TV (Karve and Hayward 2010).

Insulin resistance was predicted by objectively measured moderate- and vigorous-intensity physical activity (Ekelund *et al*., 2009). The physiological is that when muscles contract quickly, plasma glucose is rapidly absorbed; however, this does not happen very often (Khan *et al*., 2019). By substituting vigorous physical activities for 30 minutes of sedentary time, you can reduce insulin sensitivity by 15% (Kawamori *et al*., 2009).

2.5.9   DIETARY PATTERN

Unhealthy eating habits, sedentary lifestyles, and decreased physical activity are

all linked to an increased risk of prediabetes (Khetan *et al*., 2018). The cornerstone of T2DM prevention is lifestyle modification that aims to improve dietary habits and increase physical activity levels (Weisman *et al*., 2018). Diet is thought to be a modifiable risk factor for T2DM (Liu *et al*., 2000).

Soft drinks have also been linked to an increased risk of T2DM due to a direct relationship with BMI (Dhingra *et al*., 2007). Consuming processed meat on a regular basis, but not other meats, may increase the risk of T2DM (Schulze *et al*., 2004). There are numerous metabolic disorders, such as obesity, hypertension, and dyslipidemia, that may contribute to increased morbidity and mortality (Kyrou *et al*., 2018).

Early intervention in the prediabetic population has been shown to reduce the risk of T2DM by 40-70% (Ford *et al*., 2008). T2DM risk was reduced in overweight adults (40-65 years old) with impaired glucose tolerance who received individualized counselling aimed at losing weight (Khetan and Rajagopalan 2018). Following the active intervention period, there will be long-term benefits for many year (Lindström *et al*., 2013).

The role of diet in the history of T1D is not fully understood, and the results are still contradictory (Lönnrot *et al*., 2017). The milk protein responsible for the development of diabetes is bovine serum albumin (BSA) (Lamb *et al*., 2015). High consumption of sugar-sweetened beverages, processed red meat, refined grains, alcohol, and wholegrain foods is associated with an increased risk of T2DM (Weickert *et al*., 2018).

Improving the quality of carbohydrates and fats in the diet appears to be beneficial (Jannasch *et al*., 2017). In addition to the quantity and quality of macronutrients in the diet, recent research has focused on specific dietary patterns (Esposito *et al*., 2017). Even after controlling for potential confounders, higher adherence to the Mediterranean diet is associated with significantly lower T2DM risk (Salas-Salvadó *et al*., 2011).

2.5.10 ASSOCIATION OF CARDIOVASCULAR DISEASE (CVD) AND PREDIABETES

Pre-diabetes may be linked to an increased likelihood of developing T2DM, and it may also be associated with an increased risk of CVD (Ford *et al*., 2010). The

prevalence and incidence of advancement to T2DM, as well as the risk of CVD, are affected by the definition of pre-diabetes used (Beulens *et al.*, 2019). WHO-IFG and HbA1C of 6.1-6.4% had the strongest correlations with cardiovascular disease (Ford *et al.*, 2010). Prediabetes states defined by HbA1C levels of 5.7-6.4% or 6.1-6.4% were linked to an increased risk of cardiovascular disease (Beulens *et al.*, 2019).

Around 24.3% were current smokers with an estimated 10-year cardiovascular event risk of around 7% (Echouffo-Tcheugui and Selvin 2021). A number of adipokines that appear to induce metabolic risk factors that contribute to diabetes and CVD (Deng and Scherer 2010). All of the risk factors of the metabolic syndrome are mediated by insulin resistance (Reaven 2005). Individuals with pre-diabetes had consistently higher risks of stroke than NGT, but not according to HbA1c definitions (Peters *et al.*, 2014).

One component of the consensus view description of the metabolic syndrome is an elevated glucose level (Alberti *et al.*, 2005). The metabolic syndrome is most commonly associated with abdominal obesity (Reaven 2005).

Individuals with prediabetes (IFG or IGT) have a higher risk of all-cause mortality when compared to those with normoglycemia (Barr *et al.*, 2009). Individuals with prediabetes are at a higher risk of hospitalization (Wen *et al.*, 2005). In order to have the syndrome in obese people, one must also possess metabolic susceptibility, which can be accorded by other factors (Danaei *et al.*, 2011). Although not precisely, the incidence of pre-diabetes and metabolic syndrome overlap (Alexander *et al.*, 2006).

In the nondiabetic population over the age of 50, approximately twice as many people have IFG plus metabolic syndrome (Bianchi *et al.*, 2011). A significant proportion of this community also has metabolic syndrome in the absence of IFG, and vice versa (Alexander *et al.*, 2006).

2.5.11 SMOKING

People who smoke regularly have a greater likelihood of developing T2DM than non- smokers (Willi *et al.*, 2007). People who smoked 20 cigarettes per day were 61% more likely to develop T2DM (Carlsson *et al.*, 2004). Individuals who smoked less than 20 cigarettes per day were only 29% more likely to develop T2DM (Carlsson *et al.*, 2004). In a cross-sectional research of 2142 healthy Europeans (25 to 41 years old),

the relationship between total combined smoking exposure and pre-diabetes revealed a higher risk of pre-diabetes in smokers compared to non-smokers (Aeschbacher *et al*., 2014).

In smokers with pre-diabetes, the association with gradually increasing odds ratio (ORs) ranged from 1.34 in smokers with 5 pack-years of exposure to 1.80 in smokers with 5 to 10 pack-years of exposure and 2.51 in smokers with more than 10 pack-years of exposure (Campagna *et al*., 2019). The risk arises as a result of insulin insensitivity caused by nicotine, one of the chemicals found in cigarettes (Willi *et al*., 2007).

Cigarette smoking is a leading preventable cause of death and morbidity worldwide (Pirie *et al*., 2013). Tobacco uses alone was responsible for 6.3% of all global disability - adjusted (Doll *et al*., 2004). Smokers have lower levels of the transcription factor peroxisome proliferator-activated receptor-gamma (PPAR), which promotes insulin sensitivity (Bergman *et al*., 2009).

The phosphorylation of this serine distillate reduces insulin signalling (Willi *et al*., 2007). There is a strong link among cigarette smoking and T2DM, independent of other diabetes risk factors (Bergman *et al*., 2009). Studies have shown that smokers have higher adiposity than nonsmokers among people with normal BMI, which is a crucial risk factor for diabetes (Larsson *et al*., 2005).

Smoking has been linked to negative changes in body composition, which may result in the onset of diabetes (Fujiyoshi *et al*., 2016). Nicotine receptors can be found on pancreatic insulin-producing –cells (Yoshikawa *et al*., 2005). Not only does chronic nicotine exposure increase the likelihood of T2DM, but acute nicotine exposure reduces insulin sensitivity (Hur *et al*., 2007). T2D, like smoking, is one of the most significant risk factors for worldwide disease burden (Patja *et al*., 2005).

A pro-diabetic effect may be a prospective mediator for some of smoking's negative effects, but the causality of this connection has not been well formed, and its mechanisms underlying are poorly understood (Chiolero *et al*., 2008). Discovered the link among smoking and diabetes (Chen *et al*., 2008). The large bulk of Asian men smoke. China is both the world's largest consumer and producer of cigarettes (Ko *et al*., 2001). There was a significant correlation between smoking and a higher waist-to-

hip ratio in 513 Japanese men. As a result, the effect of smoking on diabetes is likely to be significant in Asian countries (Khan *et al*., 2019).

2.5.12 ALCOHOL CONSUMPTION

T2DM is a major public health issue in developed countries (Wild *et al*., 2000). Alcohol consumption has been studied as a possible modifiable risk factor for T2DM because of its impact on insulin secretion and sensitivity (Cullmann *et al*., 2012). When consumed in excess of a certain amount, alcohol is yet another risk factor for T2DM (Knott *et al*., 2015).

There was a decrease in the likelihood of T2DM when alcohol was digested in amounts less than 63 g/day (Doria *et al*., 2008). Moderate alcohol consumption may be linked to a lower risk of diabetes, whereas heavy alcohol consumption may be linked to a greater risk (Khan *et al*., 2019). There is a significant correlation between the risk of T2DM and increasing alcohol consumption above 63 g/day (Knott *et al*., 2015).

2.5.13 GENETIC FACTORS

Genetics constitutes another substantial risk factor for T2DM. Multiple investigations have indicated that individuals from specific ethnic backgrounds exhibit a higher likelihood of developing T2DM compared to those from different ethnic groups (Park *et al*., 2011). Indians living in Western countries are twice as likely as native Europeans to develop T2DM (Horikaw *et al*., 2000). People who have T2DM in one's parents are six times more likely to develop T2DM (Khan *et al*., 2019). A large number of studies have been carried out over the last 35 years to determine whether genetics is a sensible risk factor for T2DM (Horikaw *et al*., 2000).

2.6 ADIPOKINES

Adiponectin is predominantly released by adipocytes and seems to function as a hormone with both pro-inflammatory and anti-inflammatory properties in vitro and in animal models. Additionally, it may play a role in enhancing beta-cell function and survival (Kadowaki *et al*., 2006). Obesity-related insulin resistance describes T2DM (Pajvani and Scherer 2003). One such factor is adiponectin (also known as ADIPOQ, APM1, and Acrp30) (Arner 2003).

Reduced adiponectin levels serve as a precursor to and predictor of T2DM, and elevating plasma adiponectin levels enhances insulin sensitivity, likely by promoting fatty acid oxidation through AMP kinase (Tomas et al., 2000). The predominant factors contributing to the increasing prevalence of obesity in South Asians are demographic shifts, dietary patterns, and lifestyle choices, all set against a genetic backdrop (Palit *et al*., 2021). The concentration of visceral adipose tissue (AT) has been recognized as a significant risk factor for T2DM (Misra and Shrivastava 2013).

Adiponectin levels appear to be reduced prior to the onset of type 2 diabetes (Spranger *et al*., 2003). A carboxylase could be important in adiponectin signaling (Tomas *et al*., 2002). The prominent metabolic outcomes of these occurrences involve a decline in hepatic glucose production and the mechanisms through which adiponectin enhances insulin sensitivity (Yamauchi et al., 2003). Adiponectin stands out among adipokines as its systemic levels decrease with obesity, whereas the majority of adipokines are released in larger quantities as cell size increases (Turer and Scherer 2012).

In human adipose tissue, there are unidentified cells that release resistin. Consequently, resistin is not classified as a genuine adipokine in humans, and its influence on insulin resistance is less significant than previously thought based on the research (Arner 2005). Resistin may cause insulin resistance in rats in a variety of abstract ways. One technique is to increase glycogen synthesis. Another method is to inhibit adipocyte differentiation (Wolf 2004).

In humans, there exists a negative correlation between in vivo insulin levels and the rates of plasma or adipocyte adiponectin production (Altman *et al*., 2002). Adiponectin insufficiency is an impartial risk factor for the onset of type 2 diabetes (Arner 2005). For many years, it was assumed that the relationship between both the pancreas and adipose tissue was indeed (Dunmore and Brown *et al*., 2013).

Due to the adipokines (pro-inflammatory and anti-inflammatory) that it secretes, adipose tissue is a key regulator of metabolic homeostasis (Altman *et al*., 2002). Obesity distorts the fine-tuned balancing act of pro- and anti-inflammatory adipokines, causing a variety of metabolic disorders (Pramanik *et al*., 2018). One such calibrator is adiponectin, which is plentifully conveyed in white adipose tissue (Lara-Castro *et al*., 2006).

Adipsin was the initial protein recognized as being released by adipocytes. Utilize advanced technology to bring together macrophages and endothelial cells within the stroma of adipose tissue (Arner 2005). A few cell types in adipose tissue produce some adipokines (Trayhurn and Wood 2004). Obesity lessens plasma adiponectin levels despite rising adipose mass (Pajvani *et al*., 2003).

ADIPOR1 and ADIPOR2 were expected to possess seven transmembrane domains, 67% similarity with the mouse gene, and significant conservation of membrane-spanning domain names from yeast to mammals (Trayhurn and Wood 2004). The Metabolic Syndrome Era represents a novel generation of diseases that has impacted the human population in recent decades (Misra and Shrivastava 2013).

Obesity and type 2 diabetes are common, and there is a trend of prevalence based on race (Palit *et al*., 2021). Adiponectin levels are decreased in cardiovascular disease and a wide range of metabolic disorders including such obesity, inflammatory states, IR, and Type 2 diabetes (Herder *et al*., 2007). Hypoadiponectinemia is linked to the onset of IR and Type 2 diabetes (Saltevo *et al*., 2008).

ADIPOR1 seems to have a stronger affinity for the globular form of adiponectin compared to ADIPOR2, which appears to bind to the full-length form (Kharroubi et al., 2003). Both ADIPOR1 and ADIPOR2 were found to be abundantly expressed in human and rat pancreatic cells, with their expression elevated upon exposure to the free fatty acid oleate (Wang et al., 2004). The majority of these studies indicate that adiponectin levels in T2DM are generally low, even in the early stages of the disease, such as at diagnosis (Saltevo *et al*., 2008).

Imbalances in energy within additional metabolic organs like the liver, muscles, and adipose tissues lead to the aforementioned conditions (Kim et al., 2019). Secretory peptides or proteins originating from metabolic organs facilitate communication with each organ (Oh et al., 2016). Secretory factors from adipose tissues and hepatokines from the liver are essential for metabolic adaptation in T2DM (Kim *et al*., 2019).

Always a handful of these proteins have been connected to insulin sensitivity regulation (Trayhurn *et al*., 2004). Adipokines play a role in influencing the mechanisms through which they can impact insulin action. The majority of the

discussions on adipokines, as in various biomedical fields, appear to be based on animal studies (Path et al., 2001). Polymorphisms in the genes of adiponectin receptors could heighten the risk of T2DM by diminishing insulin sensitivity (Trayhurn *et al*., 2004).

The ratio of serum HMW adiponectin to total adiponectin has a higher correlation with plasma glucose levels than each of the forms separately (Vionnet *et al*., 2000). The adiponectin gene (ADIPOQ/APM1/GBP28) locus, 3q27, has been strongly linked with a number of metabolic disorders such as obesity, dyslipidemia, and T2DM (Kissebah *et al*., 2000). Because of the high fluidity of the ADIPOR1 gene, which is also discovered in skeletal muscle and pancreatic beta-cells, and the affirmation that the globular isoform plays a crucial role in mediating adiponectin action (Tomas *et al*., 2002).

ADIPOR1 is found at 1q32.1 and is telomeric to the 1q21-q24 correlation signals (Wu *et al*., 2003). A number of prospective studies have discovered that high levels of making the rounds adiponectin are linked to a lower risk of T2DM (Yamauchi *et al*., 2003). Among these secretory variables, adipokines survive out because they contribute significantly to the shared pathobiology of T2DM and obesity (Kocot *et al*., 2017).

Obesity is defined as abnormal body fat accumulation. Adipokines have such a direct effect on its advancement and severity (Lee *et al*., 2019). Excess adiposity, which is connected to dysregulated adipokine affirmation, creates adipocyte dysfunction, inflammation, and peripheral and the whole insulin sensitivity (Jung and Choi 2014). A few studies on various races have discovered a link in between SNPs in the adiponectin gene and T2DM (Vasseur *et al*., 2002).

Identifying that T2DM is a multi-factorial and polygenic metabolic illness, substantial variants in the genetic structure of T2DM among various ethnic groups have been recognised (Mori *et al*., 2002). Adiponectin has been the most widely studied adipokine in aspects of insulin sensitivity regulation (Fasshauer and Paschke 2003). Adiponectin is the most extensively studied adipokine in terms of insulin sensitivity regulation (Arner 2005).

The results of epidemiological studies that looked at the relationship between

making the rounds leptin levels and the onset of T2DM were blended (Fasshauer and Paschke 2003). ADIPOQ is composed of two introns and three exons that code for the 30 kDa adiponectin protein (Takahashi *et al*., 2000). In both animal models and humans, the adipokine exhibits evident in vivo effects on plasma glucose levels and insulin sensitivity (Arner, 2005).

In both lipodystrophy and obesity, a C-terminal fragment is found to reverse in vivo insulin resistance (Berg et al., 2001). Adiponectin-null mice exhibit insulin resistance. Initially categorized as an adipokine, resistin was identified in adipocytes, and the protein is associated with reduced glucose tolerance and the induction of hepatic insulin resistance (Steppan et al., 2001). In various obese mouse models, resistin mRNA levels decline, but gene expression increases after exposure to an insulin sensitizer. The absence of gene and protein expression in human fat cells holds significant clinical implications (Rajala et al., 2003).

Leptin is a peptide hormone primarily secreted by white adipose tissue. Its expression and release increase in large adipocytes, even when fat cell volume is normalized, suggesting that its regulation is akin to that of pro-inflammatory immune mediators in adipocytes (Otero *et al*., 2005).

Leptin has been a subject of extensive research since its replication in 1994. These effects may exert an indirect influence on insulin sensitivity (Unger *et al*., 2003). Non-adipose tissue lipid oxidation stimulation, including the liver and skeletal muscle, has an indirect impact on insulin sensitivity and would seem to be mitigated by changes in AMP activated protein kinase and lipogenic enzymes (Oral *et al*., 2002).

Leptin also restricts endocrine function, reproduction, and immunity, among other items (Skurk *et al*., 2007). There really are proinflammatory and anti-inflammatory adipokines; the aforementioned promotes inflammation and insulin resistance, whereas the latter is defensive and beneficial (Kocot *et al*., 2017). Pathogenic adjustments are caused by a mismatch of proinflammatory and anti-inflammatory adipokines (Kim *et al*., 2019).

Individuals with obesity and type 2 diabetes exhibited changes in their adipokine profile, leading to noteworthy alterations in metabolic risk and insulin sensitivity (Lee *et al*., 2019). Despite evidence that isomeric adiponectins are more pharmacologically

active than larger adiponectins, the role of adiponectin isozymes is unidentified (Fruebis *et al*., 2001). Changes in plasma adipokines and/or inflammatory parameters in T2DM are a consequence of either an excess of adipose tissue mass or are directly associated with the diabetic condition (Fried *et al*., 1998).

2.7 ADIPONECTIN GENE POLYMORPHISM

Adiponectin was identified by Scherer, P.E *et al*., (1995), which was found in the mid-1990s and has four distinct domains, an N-terminal signal peptide, a varying domain, a collagen fibres domain, and a C-terminal C1q-like globular domain. Adiponectin does have 244 amino acids and is discovered on chromosome 3q27, a region connected to type 2 diabetes and CVD (Das *et al*., 2001).

Even though the exact pathogenesis of T2DM is unknown, it is widely accepted that T2DM is a multifactorial disorder due to genetic polymorphisms and a variety of environmental factors (Khan *et al*., 2017). Adiponectin is primarily synthesised as a monomer in white adipose tissue (Vionnet *et al*., 2000). The adiponectin gene (ADIPOQ) is associated with obesity, insulin resistance, and metabolic characteristics that contribute to the development of T2DM (Tsai *et al*., 2014). Adiponectin defect is connected to coronary artery disease and high blood pressure (Sattar *et al*., 2006).

Adiponectin represents a hyperglycemic state and sheds some light on the pathophysiology of diabetes-related cardiovascular disease (Iwashima *et al*., 2004). T2DM, a rare form with serious complications, is a global public health issue (Alimi *et al*., 2021). Based on the most recent International Diabetes Federation (IDF) statistics, roughly 500 million people worldwide have diabetes that has been confirmed or given a diagnosis (Alharb *et al*., 2021).

The large percentage of diabetic patients (approximately 90% of DM cases) have type 2 diabetes, which is induced by insulin resistance in peripheral tissues, and diabetes affects mainly obese adults (Alfaqih *et al*., 2018). Prediabetes is a stage that occurs before T2DM. In prediabetes, insulin resistance is compensated for by higher serum insulin levels (American Diabetes Association 2009).

Endothelial dysfunction, enhanced carotid intima-media thickness (IMT), and coronary artery disease all are affiliated with hypoadiponectinemia (Dullaart *et al*., 2010). Macrophage foam cell transition and smooth muscle cell proliferation and

migration, each of which defend against atherosclerosis (Pilz *et al*., 2005). By 2045, the disease will have affected approximately 693 million people (Alimi *et al*., 2021). Even though insulin resistance is the predominant common characteristic of T2DM patients, some degree of insulin resistance has been noted in nondiabetic individuals (Alharb *et al*., 2021).

Prediabetes is a stage wherein active effective interventions such as a healthy diet and exercise can delay or stop the disease from progressing to "frank" diabetes (Tabák *et al*., 2012). Early biochemical changes are associated with this stage of the disease are crucial in determining individuals at risk for developing T2DM and who would advantage from the above preventive interventions (Alfaqih *et al*., 2018).

Lower serum adiponectin levels are an additional CVD risk factor in type 2 diabetes patients (Ezenwaka and Kalloo 2005). The involvement of adiponectin gene variants in T2DM patients' increased Coronary Artery Disease (CAD) risk (Al-Daghri *et al*., 2011). Diabetes-related insulin resistance is triggered by a combination of environmental and genetic factors (Cui *et al*., 2020). Risk factors for T2DM include weight gain, lack of activity, a high fat diet, hypertension, and glucose tolerance problems (Murea *et al*., 2012).

SNP45T > G and SNP276G > T of the adiponectin gene were found to be associated with CAD risk in T2DM individuals, and SNP45T > G was discovered to be linked with CAD risk (Al-Daghri *et al*., 2011). SNP45T > G and CAD risk were found to be significantly related. These finding suggest that adiponectin gene variations make a contribution to diabetics' enhanced risk of CAD (Liang *et al*., 2019). Genetic factors could play a role in the development of the illness in diverse demographics (Cui *et al*., 2020).

Individuals who really are genetically programmed to T2DM have a higher chance of developing the disease than the others (Mohammadzadeh *et al*., 2016). A few polymorphisms have been linked to the likelihood of T2DM (Cui *et al*., 2020). Growing evidence demonstrates the significance of adiponectin gene polymorphism in the advancement of T2DM (Menzaghi *et al*., 2007). Adiponectin, an adipose tissue- extracted protein (30 kDa), has been found to be lower in patients with metabolic syndrome, such as T2DM and insulin resistance (Alimi *et al*., 2021).

Diabetic patients who have elevated plasma adiponectin levels have a greater risk of serious adverse cardiovascular events (MACE) (Hung *et al*., 2010). High adiponectin plasma levels are associated with MACE in CAD patients with T2DM but not in others without diabetes (Liang *et al*., 2019). Adiponectin is an adipokine protein secretory by adipocyte cells that governs lipid metabolism, insulin sensitivity, and blood sugar levels (Ziemke and Mantzoros 2010). Regardless of the fact that plasma adiponectin levels were lower in T2DM and CAD patients (Wannamethee *et al*., 2007).

To counteract the atherosclerotic process, vascular inflammation stimulates adiponectin synthesis (Fujita *et al*., 2015). Adiponectin levels are influenced by age, gender, and BMI, with obese people having the lowest levels. Its level is low in T2DM (Jiang *et al*., 2018). Adiponectin levels reflect an increased risk of cardiovascular and mortality, which appears to be related to disease severity (Liang *et al*., 2019). After attempting to control for traditional atherosclerosis risk factors, adiponectin was found to be negatively associated with the intensity of peripheral arterial disease (Jiang *et al*., 2018).

Anti-inflammatory, anti-atherosclerotic, and anti-diabetic characteristics of adiponectin (Hossain *et al*., 2017). Adiponectin levels found in the blood are also lowered in T2DM patients (Alimi *et al*., 2021). ADIPOQ, an adiponectin-coding gene located on chromosome 3q27, has indeed been suggested as a genomic locus for T2DM using genomic sequence scans (Moher *et al*., 2009). The connection of mainly two single nucleotide polymorphisms (SNPs) of the ADIPOQ gene, rs266729 and rs1501299, with the risk of T2DM has been studied in various populations all over the world (Alimi *et al*., 2021).

Treatment with the thiazolidinedione (TZD) class of insulin-sensitizing drugs can significantly improve endothelial and adipose tissue dysfunction by continuing to increase adiponectin expression of genes and secretion (Rizza *et al*., 2012). The advancement in insulin resistance and diabetes caused by pioglitazone is facilitated, at least in part, by an adiponectin-dependent pathway (Dormandy *et al*., 2005). Adiponectin may play a key role in TZDs' cardiovascular protective role (Kubota *et al*., 2006). Serum adiponectin levels were discovered to be lower in prediabetic people from various populations (Lai *et al*., 2015).

The adiponectin gene (ADIPOQ) encodes adiponectin and is discovered on chromosome 3q27 (Hossain *et al*., 2017). There is currently no recombinant adiponectin protein that's also widely used in the field trials (Liang *et al*., 2019). The biomarkers that precede insulin resistance, a cornerstone of prediabetes, provide fertile soil for the identifying of prediabetes disease biomarkers (Alfaqih *et al*., 2018). The interactions between adiponectin single-nucleotide polymorphisms (SNPs) and elements have not been investigated thoroughly (Cui *et al*., 2020).

Resistin is a recently found hormone specific to adipose tissue that straight induces insulin resistance in muscles and the liver (Steppan *et al*., 2001). Resistin helps to stimulate the expression of endothelin-1 messenger RNA in endothelial cells, making a contribution to endothelial dysfunction (Verma *et al*., 2003). Enhances the expression of the cellular adhesion molecule VCAM-1 and MCP-1, which are both involved in the development of early atherosclerotic lesions (Degawa-Yamauchi *et al*., 2003).

Though the proinflammatory pathways, resistin promotes cardiovascular disease. Resistin serum concentrations are higher in obese individuals as well as T2D patients (Youn *et al*., (2004). In this condition, resistin is also high. Moreover, in this population, diabetic nephropathy is a substantial predictor of subsequent cardiovascular disease (Burnett MS *et al*., 2006). Low plasma adiponectin levels could play a role in the onset of insulin resistance and T2DM (Medina-Bravo *et al*., 2011). ADIPOQ gene SNPs affiliated with varying levels of adiponectin". "SNP rs266729 (11377 C > G) and rs1501299 (+276 G > T) in the proximal promoter of ADIPOQ gene has been studied extensively (Wang *et al*., 2009).

Adiponectin levels seem to be commonly lower in obese individuals and individuals with T2DM (Spranger *et al*., 2003). T2DM patients who evolved restenosis after being stented with a bare metal stent (BMS) had higher serum resistin concentration levels than those who did not (Liang *et al*., 2019).

The ADIPOQ gene rs1501299 SNP is linked to polycystic ovarian syndrome (PCOS), a disease strongly associated with insulin resistance (Alfaqih *et al*., 2018). SNP rs266729 in the ADIPOQ promoter region causes an amino acid change that ultimately resulted in the replacement of cytosine with guanine at nucleotide position -11377 (11377C > G) and is connected to T2DM (Hsiao *et al*., 2016).

Although the G allele of the ADIPOQ rs266729 SNP would seem to be important in affiliations with T2DM risk in various populations, genetic evidence of its effect on T2DM has indeed been ambiguous (Prior *et al*., 2009). Serum resistin could be a biological marker for the development of cardiovascular disease in patients who have T2DM and restenosis within a week of bare metal stent implantation (Liang *et al*., 2019). The role of resistin in the development of atherosclerosis in T2DM patients (Schäffler *et al*., 2005). The therapeutic potential of resistin antibodies in T2DM for the avoidance of diabetic cardiovascular problems and restenosis to bare metal stents (Schäffler *et al*., 2005).

2.8. RESISTIN AND ITS SNP

Resistin, an adipocytokine, has indeed been associated with obesity and diabetes (D'Aiuto *et al*., 2004). Resistin is a protein hormone that is made by adipocytes in addition to immunocompetent cells, such as those discovered in adipose tissue (Boumaiza *et al*., 2012). Resistin impacts glucose tolerance and insulin action, and thus adds to the pathophysiology of obesity and insulin resistance in humans (Chu *et al*., 2008 and Wang *et al*., 2009). Not only does resistin contribute to insulin resistance, but it also aids in the inflammatory process via pro-inflammatory agents. Resistin and periodontitis have a bidirectional relationship (Rode *et al*., 2019).

The G/G genotype at SNP-420 increased T2D vulnerability by rising promoter activity via Sp1/3 transcription factor binds directly (Osawa *et al*., 2007). The fact that resistin is conveyed by polymorphonuclear leukocyte cells and macrophages tries to explain why resistin levels have risen with periodontitis (Gokhale *et al*., 2014).

Resistin levels have indeed been discovered to be significantly higher in both genetic and diet-induced obesity models (Rathwa *et al*., 2019). SOCS-3 activation causes the degradation of Insulin Receptor Substrate 1/2 (IRS1/ 2) as well as the induction of insulin resistance (Steppan *et al*., 2005). Resistin levels have been found to impede the insulin signalling pathway (Rathwa *et al*., 2019). RETN, the human resistin gene, is found on chromosome 19p13.3.6. In living beings, the RETN prepeptide is 108 amino acids extensive (Boumaiza *et al*., 2012).

Plasma resistin levels were found to be highest in the G/G genotype, followed by the C/G and C/C genotypes (Osawa *et al*., 2010). SNP-358 (rs3219175), which is

also discovered in the RETN promoter region, is required for a G at SNP-420 to confer the highest plasma resistin stages (Onuma *et al.*, 2010).

SNPs in the resistin gene (RETN) would affect plasma concentrations of resistin and could be linked to inflammatory conditions like diabetes and periodontal disease (Lau *et al.*, 2011). In healthy subjects, increased resistin predicted the presence and severity of coronary artery disease (Norata *et al.*, 2008). The role of resistin in MetS is still debated (Steppan *et al.*, 2001).

Some research findings found a link between resistin levels and obesity and diabetes, whereas others discovered no link between resistin levels and metabolic markers (Reilly *et al.*, 2005 and McTernan *et al.*, 2006). This inconsistency is heavily influenced by ethnicity (Ervin *et al.*, 2009). The position of resistin in the pathogenesis of human obesity and diabetes urged genetic studies in diverse demographics (Engert *et al.*, 2002).

RETN promoter SNPs have been discovered to increase T2DM susceptibility by continuing to increase circulating resistin levels (Osawa *et al.*, 2004). RETN promoter SNPs have been discovered to increase T2D susceptibility by continuing to increase circulating resistin levels (Norata *et al.*, 2008). Elevated concentrations of circulating resistin may well be connected to metabolic syndrome and atherosclerosis via insulin resistance (Ohmori *et al.*, 2005).

A positive relationship between serum and Gingval Crevicular Fluid resistin levels and all clinical parameters (Rode *et al.*, 2019). It is still debatable whether circulating resistin or SNP- 420 is connected to insulin resistance or T2DM in humans (Schwartz *et al.*, 2011). American subjects with increased serum resistin levels had a higher likelihood of developing T2DM (Chen *et al.*, 2009). The G/G genotype at SNP-420 anticipated glycemic progression (Xu *et al.*, 2007).

Genetic factors as well as several single-nucleotide polymorphisms in the RETN gene can explain up to 70% of the variation in making the rounds resistin levels (Azuma *et al.*, 2004). RETN rs1862513 has been connected to the regulatory oversight of RETN gene expression and serum resistin levels (Osawat al 2004). High levels of resistin in the blood have been linked to an increased risk of obesity, insulin resistance, and T2DM (Santilli *et al.*, 2016).

T2DM, RETN promoter polymorphisms, and nonalcoholic fatty liver disease, chronic kidney disease, CAD, polycystic ovary syndrome, and hypertrophic cardiomyopathy are all linked to T2DM (Tang *et al*., 2008). Several prior genome-wide association studies have not recognised RETN SNP-420 as a T2DM susceptibility locus (Onuma *et al*., 2010). One of the epigenetic mechanisms for regulating transcription is DNA methylation, which may be influenced by environmental factors (Suzuk *et al*., 2008).

The RETN rs1862513 polymorphism has been associated with obesity, insulin sensitivity, T2DM and cerebrovascular disease (Kunnari *et al*., 2005). The rs3745368 SNP in the 3'UTR of the resistin gene may impact resistin gene expression, which in turn affects the risk of developing diabetes and hypertension (Tan *et al*., 2003). This disparity could be explained by factors such as epigenetic effects and ethnic differences in genome (Onuma *et al*., 2010). Critical mediator between both the genome and the surroundings. A prospective DNA methylation site is a cytosine-phosphate-guanine (CpG) dinucleotide (Lister *et al*., 2009).

In these CpG dinucleotides, DNA methylation occurs primarily at a cytosine. Because promoter methylation is linked to decreased transcription, promoter SNPs that affect CpG sequences could affect gene expression (Bird 2007). It has been proved that DNA methylation influences the pathogenic mechanisms of T2DM by influencing insulin secretion and insulin resistance (Yang *et al*., 2012).

2.9 LEPTIN AND ITS SNP

Leptin is an adipokine hormone that is primarily secreted by adipocytes (Denroche *et al*., 2014). Leptin regulates body fat by suppressing appetite, improving energy expenditure, and trying to regulate glucose homeostasis (Amitani *et al*., 2013). Leptin is an adipokine that is primarily produced in adipocytes and restricts energy expenditure and food intake (Pan *et al*., 2014).

Leptin is involved in the pathophysiology of obesity and may impact serum insulin levels and the development of T2DM (Lakka *et al*., 2000). Leptin has been implicated in the pathogenesis of diabetes (Wannamethee *et al*., 2007). Leptin lessens insulin synthesis and secretion while increasing hepatic insulin extraction. It has also been discovered that leptin boosts insulin sensitivity (Adiga *et al*., 2017).

Genetic influences on bodyweight control are carried out via various pathways, including many hormones (Friedman 2004). Leptin is a polypeptide hormone released by adipose tissue that resembles cytokines in structure (Gahagan 2020). Serum leptin can represent adipose tissue energy stores, reflecting total body fat (Noriko *et al.*, 2008). LEP and LEPR genes are highly polymorphic, and a number of SNPs in these two genes have been identified as adjustment for potential confounders to the pathophysiology of obesity and diabetes (Hoffstedt *et al.*, 2002).

The LEP G2548A SNP has been linked to T2DM and metabolic traits in the majority of studies (Meshkani *et al.*, 2016). The LEPR Q223R SNP has been linked to an increased risk of T2DM (Ying *et al.*, 2009).". In animal models, the gene that encodes leptin (LEP) was found to be associated with obesity, and LEP was initially identified as the obese gene (Campfield *et al.*, 1995). Serum leptin levels were found to be higher in obese individuals (Hijjawi *et al.*, 2018).

Resistance to leptin, rather than absolute levels, is a major feature of human obesity (Mazahreh *et al.*, 2019). Insulin, which is frequently elevated in obese people, stimulates leptin secretion (Amitani *et al.*, 2013). Diabetes mellitus is not linked to leptin levels (Adiga *et al.*, 2021). There is a significant positive relationship between plasma leptin levels and diabetes (Sun *et al.*, 2010).

The LEPR gene is found on chromosome 1p31 and encodes a 1165 amino acid long single transmembrane protein that is found in a variety of tissues (Mantzoros *et al.*, 1998). Because the LEPR gene is also known as the T2DM gene, LEP and its receptor, LEPR, may be among the relevant and interesting genes involved in the etiopathogenesis of T2DM (Li *et al.*, 2017).

LEP and its receptor, LEPR, could be among the interesting and relevant genes involved in the etiology of T2DM (Liang *et al.*, 2004). Multiple human studies confirmed the role of both the gene and its receptor in the development of obesity, while other reports found no link (Bender *et al.*, 2011).

Individuals with prediabetes are frequently obese, and their serum insulin levels are elevated to compensate for insulin resistance (Aljanabi *et al.*, 2021). The link between the LEP G2548A and LEPR Q223R variants and higher BMI (Shen *et al.*, 2014).

Obesity has been linked to the combination of LEP G2548A and LEPR Q223R polymorphisms (Duarte *et al*., 2007). G2548A (rs7799039) is a single nucleotide polymorphism (SNP) in the promoter of the leptin gene, with a G to A substitution at nucleotide -2548 upstream (Wang *et al*., 2020).

T2DM is associated with coronary artery disease and the development of cancer, with high leptin levels being a source of contention (Yang *et al*., 2019). Elevated leptin levels are caused by the upregulation of the leptin gene as a result of insulin resistance and hyperinsulinemia (Laivuori *et al*., 2000). It has been established that leptin inhibits insulin secretion (Adiga *et al*., 2021).

Hypothesis for Research

Null Hypothesis (Ho):

There is no significant correlation between single nucleotide polymorphism in adipokines genes and the plasma level of adipokines in individuals with prediabetes.

Alternate hypothesis (Ha):

There is a significant correlation between single nucleotide polymorphism in adipokines genes and the plasma level of adipokines in individuals with prediabetes

OBJECTIVES OF THE STUDY

- To evaluate single nucleotide polymorphism in adipokine genes and estimate serum adipokine level in individuals with prediabetes.

- To establish the correlation between single nucleotide polymorphism in adipokines genes and plasma level of adipokines in individuals with prediabetes.

- To correlate insulin resistance with adipokine level in individuals with prediabetes

CHAPTER-3

MATERIALS AND METHODS

3.1 RESEARCH DESIGN AND APPROACH

A case-control study layout was adopted for the present study to identify the SNP polymorphism in adipokine genes and also its association with plasma concentration of adipokines among prediabetes subjects. Study carried out in rural and urban areas in Kerala between January 2020-2021.

A well-defined proforma was used to record the details of study subjects regarding their demographic, clinical and biochemical parameters. Anthropometric measurements such as weight and height have been documented to assess the body mass index and obesity. Informed consent from subjects and ethical clearance was produced prior to study.

3.2 SAMPLE

A case-control study conducted in Aster Mims Hospital, Kerala with 150 prediabetes subjects (40 obese and 110 non-obese) and 150 controls; age group between 30-50 years of both genders.

Insulin level will be measured by ECLIA and insulin resistance (IR) by Homeostasis Model Assessment (HOMA-IR), calculated as glucose (mg/dl) x insulin (µU/ml)/405nm. HbA1C level to screen prediabetes by HPLC method.

- A 2hrs oral glucose tolerance test (OGTT) was performed to rule out prediabetes. OGTT using 75 g of glucose was performed according to WHO criteria:
    - IFG (fasting plasma of 110-126mg/dl) and 2hrs post glucose load less than 140mg/dl
    - IGT (fasting plasma glucose ≤110mg/dl) and 2hrs post glucose load of 140-200mg/dl
- Elder subjects with diabetes and other serious physical or mental illness were excluded.
- Elder subjects with normal glucose tolerance was matched with prediabetes subject with same gender with similar criteria.
    - Blood sample for glucose analysis include collecting and storing the blood in evacuated tube containing sodium fluoride (glycolytic inhibitor) and potassium oxalate (anticoagulant). Plasma glucose was determined by the

glucose oxidase-peroxidase method.

- o A total of 5ml blood was collected from each subject and separated into 2 containers: transferred 2ml blood to EDTA tubes for analysing DNA and the remaining 3ml for serum isolation and further biochemical analysis.

- o The blood sample was used for DNA isolation and plasma sample for adipokine estimation.

- o Measurement of adipokines (adiponectin, leptin, and resistin) was measured by ELISA.

- o DNA was extracted from EDTA whole blood sample. Gene polymorphism will be assessed by real-time PCR. The genotyping result for each SNP marker was verified using DNA sequencing.

3.3 STATISTICAL ANALYSIS

Statistical analysis was performed using IBM SPSS version 21.0 software (Chicago USA). Numerical variables were represented using mean and standard deviation. To test the statistical significance of the comparison of demo and biochemical parameters, Adiponectin, Leptin and Resistin level between obese, non-obese and control group and also the comparison of Adiponectin, Leptin and Resistin level between their respective polymorphisms, one way ANOVA followed by multiple comparison Bonferroni test was applied. Allelic and genotype frequencies were presented with 95% confidence interval (C.I) and were analysed using chi-square test.

3.4 SAMPLE COLLECTION CRITERIA

- ❑ Study Group 150 prediabetes subjects (40 obese and 110 non-obese). age group between 30-50 of both sexes. A detailed assessment that includes Demographic Data (age, sex, height, weight, etc.), family history, and medical history was taken. Insulin level was measured by ECLIA and insulin resistance (IR) by Homeostasis Model Assessment (HOMA-IR), calculated as glucose (mg/dl) x insulin (µU/ml)/405nm. HbA1C level to screen prediabetes by HPLC method. A 2hrs oral glucose tolerance test (OGTT) was performed to rule out prediabetes.

- ❑ Control Group age group between 30-50 of both sexes. A detailed assessment that includes Demographic Data (age, sex, height, weight, etc.), family history, and medical history was taken. Insulin level was measured by CLIA and insulin resistance (IR) by Homeostasis Model Assessment (HOMA-IR), calculated as glucose(mg/dl) x insulin (µU/ml)/405m. HbA1C level to screen non-prediabetes by HPLC method.

A 2hrs oral glucose tolerance test (OGTT) was performed to rule out non-prediabetic subjects.

- ❑ Inclusion Criteria

  - Age group between 30-50 of both sexes.

  - IFG (fasting plasma of 110-126mg/dl) and 2hrs post glucose load less than 140mg/dl

  - IGT (fasting plasma glucose ≤110mg/dl) and 2hrs post glucose load of 140-200mg/dl

- ❑ Exclusion Criteria

  - Elder subjects with diabetes and other serious physical or mental illness.

  - Those not willing to participate in the study.

  - The same exclusion criteria are applied for the control subjects also.

3.5 ETHICAL CONSIDERATION

The study was started after getting the ethical clearance and permission was granted by the Institutional Ethics Committee (reg no: EC/NEW/INST/2019/406 & ECR/301/Inst/KL/2013/RR-19; Dated June 2020). Written informed consent was obtained from both test and control groups.

3.6 DATA COLLECTION PROCEDURE

3.6.1 Blood Sample Collection

All participants in the study were asked to fast overnight. Sample for fasting blood sugar and HbA1C was taken.

➢ Blood sample for glucose analysis include collecting and storing the blood in evacuated tube containing sodium fluoride (glycolytic inhibitor) and potassium oxalate (anticoagulant). Plasma glucose was determined by the glucose oxidase-peroxidase method.

➢ After selection of study subjects, from them a total of 5ml blood was collected from each subject and separated into 2 containers: 2ml in an EDTA coated tube for DNA analysis and 3ml of serum isolation and further biochemical analysis. All samples were stored at -20^0 C before use.

The following laboratory investigations were performed:

- Fasting Blood Sugar (GOD-POD Method)
- HbA$_1$C
- Oral Glucose Tolerance Test
- Insulin
- Human Resistin
- Human Adiponectin
- Human Leptin
- Real time PCR
- DNA sequencing

3.6.2 **Estimation of Fasting Blood Sugar (GOD-POD Method)** (Trinder's 1969)

This method was done using GENX Glucose estimation kit.

PRINCIPLE: Glucose is oxidized by glucose oxidase to form gluconic acid and hydrogen peroxide. Hydrogen peroxide is then converted to water and nascent oxygen by peroxidase enzyme. In the presence of phenol reagent, the released oxygen is combined with 4 - amino antipyrine to form pink coloured complex. The color intensity is measured at 505 nm which is directly proportional to the glucose concentration present in the sample.

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

$$H_2O_2 + 4 - \text{Amino antipyrine (PAP)} + \text{Phenol} \xrightarrow{\text{Peroxidase}} 4H_2O + \text{Quinoneimine}$$
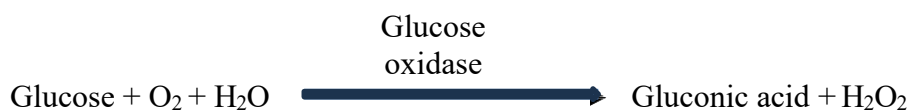
PROCEDURE

3 test tube were labelled as B, S, T for blank, standard, test respectively. 10 μL of distilled water, 100mg% (100mg/dl) of glucose standard and sample (plasma), were pipetted into B, S, T respectively. 1ml of glucose working reagent was added into all the test tubes. Mixed well and incubated for 15 minutes at 37^{0}c. Absorbance of the standard and test were measured against blank at 505 nm.

3.6.3 Estimation of HbA$_1$C (Cerami and Koenig 1978)

Glycated Hb level were measured using Cation-exchange high-pressure liquid chromatography (HPLC) on a D-10 system (Bio-Rad Laboratories, Hercules, CA, USA) using reagents according to the manufacturer's instructions. HbA$_1$C value was taken according to the National Glycohemoglobin Standardization Program (NGSP).

3.6.4 Estimation of Oral Glucose Tolerance Test (Burtis and Ashwood 1994)

After 10- 12-hour of overnight fast the blood was collected for an oral glucose tolerance test. The participants were administered the given load of glucose i.e., 1.75 g/kg body weight, up to 75 g of glucose. Venous blood sample for the individuals of plasma glucose was collected at 0,30,60,90 and 120 minutes. Plasma glucose was determined by GOD – POD method. Diabetes can be diagnosed by FPG $\geq$126 mg/dL (7.0 mmol/L) and 2-hr PG $\geq$200 mg/dL (11.1 mmol/L) (American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2018. Diabetes care. 2018 Jan 1;41(Supplement_1): S13-27.).

3.6.5 Estimation of Insulin (Ashby and Frier 1981)

PRINCIPLE: Insulin estimation was done by electrochemical luminescence immunoassay (ECLIA). It's a simultaneous one step immune enzymatic (sandwich) assay. In to the well sample is added along with mouse monoclonal anti insulin alkaline phosphatase conjugate and paramagnetic particles coated with mouse monoclonal anti insulin antibody. Insulin present in the serum or plasma binds to the antibody on the solid phase at the same time different antigenic site present on the insulin molecule reacts with the enzyme conjugate. After incubation in the well, the unbound particles are washed away and materials bounds to the solid phase are held by the magnetic field. The chemiluminescent substrate Lumi – Phos 530 is added to the well leads to the production of light which is measured by using a luminometer. The light production is directly proportional to the concentration of insulin present in the sample. By using the stored multi point calibration curve, the amount of insulin is determined.

MATERIALS REQUIRED

1. Ultrasensitive insulin calibrators (Provided at zero and approximately 1.0, 10, 50, 150

and 300 µIU/mL (7.0, 70, 350, 1050 and 2100 pmol/L).

2. Sample diluent A

3. Substrate

4. Wash buffer

PROCEDURE

Ultrasensitive insulin was determined by using the Beckmann Coulter analyser.

3.6.6 HUMAN RESISTIN, Retn GENLISA (Steppan *et al.*, 2001)

PRINCIPLE:

This technique is based on sandwich ELISA. Monoclonal Abs specific for Human Resistin, Retn has been coated on the wells of microtiter strips provided. Samples and standards are pipetted into these wells. These monoclonal antibodies are bounded by Human Resistin, Retn present in the sample. During the first incubation, biotin labelled Ab and Streptavidin – HRP is simultaneously incubated to form a complex. After washing unbounded enzymes are removed. Followed by the addition of TMB substrate induce a coloured reaction product. The color intensity was proportional to the amount of Human Resistin, Retn present in the sample. By the addition of stop solution color development was stopped and the absorbance was measured at 450 nm.

REQUIREMENTS

1. Retn Antibody Coated microtiter plate

2. Concentrated Standard, Human Retn

3. Biotinylated Retn antibody

4. Streptavidin – HRP Conjugate

5. Standard diluent

6. 30X Wash buffer

7. Substrate A

8. Substrate B

9. Stop solution

PROCEDURE

50 µL standard and 40 µL sample were added to standard and sample wells respectively. 10 µL Biotinylated Retn antibody added to sample wells and 50 µL Streptavidin - HRP conjugate added to standard and sample wells except the blank. Mixed well and plates were covered with a sealer and incubated for 60 minutes at 37^0 c. After incubation plate was washed 4 times using diluted wash buffer (1X) .50 µL of substrate A and substrate B were added to all the wells. Plate was then incubated at 37^0c for 30minutes. 50 µL of stop solution was pipetted in to all the wells. All the blue color was changed to yellow color. The absorbance was measured at 450nm.

3.6.7 HUMAN ADIPONECTIN, ADP GENLISA (Hara *et al*., 2003)

PRINCIPLE:

In this technique microwells are pre coated with specific monoclonal Abs specific for the detection of Human Adiponectin, ADP. Standards and samples are added to the microwells. The Human Adiponectin, ADP present in the sample is bounded by the Abs present in the well. Biotin labeled Ab and Streptavidin HRP is pipetted in to the microwells and incubated, leads to the formation of a complex. Unbounded Abs are removed by washing and TMB substrate is added to all the wells. The color develops depending on the concentration of Human Adiponectin, ADP in the sample. By the addition of stop solution, the color development is stopped and absorbance was measured at 450nm.

REQUIREMENTS

1. Human ADP Antibody Coated microtiter plate

2. Concentrated Standard Human ADP

3. Biotinylated ADP antibody

4. Streptavidin – HRP Conjugate

5. Standard diluent

6. 30X Wash buffer

7. Substrate A

8. Substrate B

9. Stop solution

PROCEDURE

To the microtiter plates 40 µL sample and 50 µL standard added to respective sample and standard wells.10 µL of biotinylated ADP antibody was added to sample wells. Then 50 µL of Streptavidin -HRP Conjugate were added to sample and standard wells except the blank. Microwell plates, mixed well and covered with a sealer then incubated for 60 min at 37^0c. After incubation the contents were aspirated and washed for 4 times using diluted wash buffer (1X). 50 µL of substrate A and 50 µL of substrate B were added in all the wells. The plate is then incubated for 10 min at 37^0 c. 50 µL of stop solution is pipetted in to all the wells and resulting color was measured at 450nm.

3.6.8 HUMAN LEPTIN, LEP GENLISA (Maffei *et al*., 1995)

PRINCIPLE: Sandwich ELISA technique.

Monoclonal Abs are pre coated into microwells. Samples and standards are pipetted into microwells and Human Leptin, LEP present in the sample are bound by the antibodies. Biotin labeled antibody is added and followed by Streptavidin – HRP is pipetted and incubated to form a complex. After washing microwells in order to remove any non – specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Human Leptin, LEP in the sample. Color development is then stopped by addition of stop solution. Absorbance was measured at 450 nm.

REQUIREMENTS

1. LEP Antibody Coated microtiter plate

2. Concentrated Standard, Human LEP

3. Biotinylated LEP antibody

4. Streptavidin – HRP Conjugate

5. Standard diluent

6. 30X Wash buffer

7. Substrate A

8. Substrate B

9. Stop solution

PROCEDURE: To the microtiter wells 50µL standard and 40µL of sample is added respectively. Then 10 µL of Biotinylated LEP antibody is added to respective sample wells except standard wells. 50µL Streptavidin – HRP conjugate are added to standard and sample wells except the blank. Mixed well and plates are covered by sealer and then incubated 60 minutes at 37^0 c. The wells are washed for 4 times using diluted wash buffer (1X). Then 50 µL substrate A and 50 µL substrate B were added to all the wells. The plate is incubated for 10 minutes at 37^0 c. 50 µL stop solution is added and the blue color changed to yellow color. The intensity of the color measured at 450nm.

ISOLATION OF GENOMIC DNA WITH NUCLEOSPIN BLOOD

REQUIREMENTS

1. Machery Nagel NucleoSpin Blood

2. RNase A, lyophilized, dissolve to 20 mg/ml in H2O; store at -20°C

3. Absolute ethanol

4. A tabletop microcentrifuge that can reach 11,000 x g is required.

PROCEDURE

Before starting the procedure, the elution buffer EB was preheated at 70^0 c on a heat block. Cell pellets were bringing back to room temperature (15-25^{0}c). Buffer B5 and Protein kinase K were prepared by using the kit insert provided by the manufacture. Cell pellet volume more than 200 µL was spin down and resuspended in 200 µL PBS.

The procedure includes 6 steps.

Step 1: Lysis of the cell pellet.

1. The cell pellets resuspended in 200 µL PBS were transferred to 1.5ml of microcentrifuge tube.

2. 25 µL of Protein kinase A was added and mixed using a vortex mixer.

3.   Then 1 µL of RNase A (20mg/mL) was added and mixed. (Clumpy cell pellets are incubated at 56^0 c for 5- 20 minutes and vortex the tubes for several times to further break up the cell). 200 µL of buffer B3

4.   200 µL of Buffer B3 was added and mixed vigorously for 10s. (Vigorous mixing is important for DNA yield and purity)

5.   Samples were incubated at 70^0 c in a water bath for 10-30 minutes and vortexed the solution for every 10s until the solution becomes clear.

6.   The cell lysate was cooled and proceeded for DNA precipitation.

(When hot lysate is placed on the column, the DNA yield decrease due to the evaporation of ethanol).

Step 2: Precipitation of DNA

7.   210 µL of absolute ethanol was added to each sample and vortexed.

STEP 3: Binding of DNA.

8.   To all the NucleoSpin Blood Mini Columns all the lysate was added.
While adding, avoid drops on the rim of the column. Before centrifugation, the lysate may start flow through the column. It will not affect the DNA yield or purity.

Columns are kept in an upright position

9.   The tubes were closed and centrifuged 1 min at 11,000 x g. Discarded the flow through.

STEP 4: Washing of silica membrane

10.   In the first wash 500 µL of Buffer BW was added to all the Nucleospin Mini Column and centrifuged 1min at 11,000 x g. Discarded the flow through.

11.   In the second wash 600 µL buffer B5 was added. The mixture was centrifuged at 1min at 11,000 x g. Discarded the flow through.

12.   After washing tubes are dried by centrifuging 1min at 11,000 x g. Columns are carefully removed from the rotor by avoiding the contact of flow through with the column outlet. Collection tubes are then discarded.

Step 5: Elution of DNA

13. To a new 1.5ml of microcentrifuge tube columns are directly inserted. Preheated buffer BE 100 µL was added directly to the centre of the silica membrane.

14. Tubes are then incubated overnight at 4^0c or at room temperature for 5min.

15. Contents are eluted after centrifugation at 11,000 x g for 1min.

Step 6: Dilution and storage of gDNA.

16. The concentration gDNA was measured using Nanodrop/ Qubit. Dilute the gDNA when its concentration is higher than 200ng/ µL. Actual measured concentration of the eluted DNA was used to calculate the dilutions.

17. The gDNA were stored at 4^0c for short term and for long term at -20^0c.

3.6.9 AGARAOSE GEL ELECTROPHORESIS

PRINCIPLE:

DNA molecules possess a negative charge in their backbone structure due to the presence of PO4 – groups and this is exploited for its separation as the negatively charged molecules move towards the anode on application of electric potential. Agarose gels are made between 0.7% (provides good resolution of large 5-10 kb DNA fragments) and 2% (which provides good resolution for small 0.2 – 1 kb fragments).

MATERIALS REQUIRED

▪ DNA sample
▪ Quartz cuvette
▪ Single distilled water
▪ Triple distilled water
▪ Tissue paper

PROCEDURE

The cuvettes were rinsed with triple distilled water. Base correction was done using triple distilled water. 995 µL of triple distilled water was added to 5 µL DNA sample. Absorbance

of the DNA sample was measured at 260/280nm against triple distilled water. The DNA concentration present in the diluted sample were calculated using the formula, 50 x OD x Dilution factor.

RT PCR

Genotyping is done using real time PCR (ABI 7500 instrument)

PROCEDURE:

Step 1:

DNA samples are arrayed in to 96 well PCR plate. A no - template controls (NTCs) is included on each well plate. Arrayed DNA samples are dried and taken out for genotyping reactions.

Genotype reactions are carried out by using wet DNA method.

STEP 2: Preparation of KASP genotype mix.

For 96 well reaction plate 5 µL DNA, 5 µL 2x KASP master mix and 0.14 KASP assay mix were added to each well. The final volume made up to 10 µL.

STEP 3: Dispense genotype mix on to the reaction plate

5 µL of genotype mix were pipetted in to each DNA sample in the reaction plate

STEP 4: Centrifugation.

Well plates are covered using an optically clear seal and centrifuged at ≥550 x g.

STEP 5: Run for thermal cycling

Thermal cycling was done by using the KASP thermal protocol as described in the table:

Step	Description	Temperature	Time	Number of cycles per step
1	Activation	94°C	15 minutes	1 cycle
2	Denaturation	94°C	20 seconds	10 cycles
	Annealing / Elongation	61-55°C	60 seconds (drop 0.6°C per cycle)	
3	Denaturation	94°C	20 seconds	26 cycles
	Annealing / Elongation	55°C	60 seconds	

Step 6; Read the plate and analyze the data.

Reaction plate was read in a FRET- capable plate reader (Temperature below: 40^0c) after completion of thermal cycle. Data were analysed in a KlusterCaller™ (LGC) (www.lgcgroup.com/software).

SANGER SEQUENCING

PCR/ Plasmid sequencing was done by using Sanger method. Here 1-2µl of PCR/plasmid samples were separated on a 2% agarose gel at 120V, for 60min or the samples reaches the $3/4^{th}$ of the gel. Qualified QC samples after visualizing under the UV light were taken out for PCR plate set up.

In the plate set up, DNA samples, primer and reaction mix (2 µl DNA + 1 µl Primer + 7 µl Reaction Mix) were added to the 96 well plate. Then it was covered and put into the thermocycler under proper reaction conditions and processed for PCR. The data files were analysed using sequencing analysis V5.3 software.

SNP information of adipokine gene

SNP ID	Gene Position	Location	Forward Primer Reverse Primer
rs266729	ADIPOQ11377 (C>G)	Promoter	ACTTGCCCTGCCTCTGTCTG GCCTGGAGAACTGGAAGCTG
rs7799039	LEP2548(G>A)	Promoter	5^1 - TTTCTGTAATTTTCCCGTGAG-3^1 3^1-AAAGCAAAGACAGGCATAAAAA-5^1
rs1862513	RETN420(C>G)	Promoter	5^1-TGTCATTCTCACCCAGAGACA-3^1 3^1-GGGCTCAGCTAACCAAATC-5^1

Primer sequence for the analysis of single nucleotide polymorphism of ADIPOQ gene, LEP gene and RETN gene.

Chapter-4

RESULTS

According to the level of HbA1c and OGTT, the study subjects were classified into two as control group (N=150) and pre-diabetes group (N=150). According to BMI level, the pre-diabetes group was again divided into obese (N=40) and non-obese (N=110) subjects. Firstly, the socio-demographic data of study subjects were compared with biochemical parameters which was illustrated in Table 4.1. Out of 300 study subjects, the observed mean age of control was 39.03±5.72, for pre-diabetic non-obese group it was 38.39±6.31and for pre-diabetic obese 39±5.20. However, there was no statistical significance was observed among the mean age of study subjects (p=0.78). Whereas, other parameters were showed a statistically significant difference among study subjects with a p value <0.001.
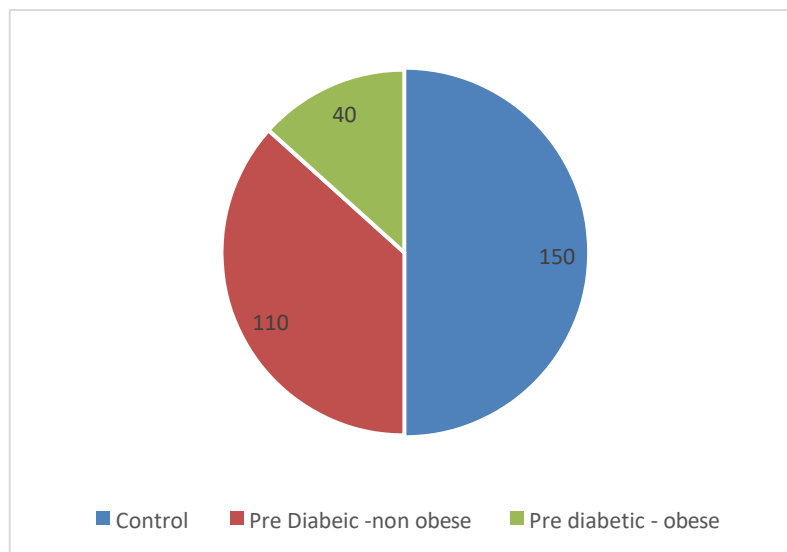
Fig. 4.1. Distribution of study subjects

Distribution of study subjects was illustrated in the fig.6.1. The study subjects 50% (N=150) were control group and remaining 50% (N=150) were pre-diabetes group. In case of the pre-diabetes group, they are again classified based on BMI, among them 26.66% (N=40) were obese and the remaining 73.34% (N=110) non-obese subjects

Table 4.1 Comparison of socio-demographic characteristics and biochemical parameters among study subjects

Parameters	Control (N=150) Mean ± SD	Pre-diabetic; Non-obese (N=110) Mean ± SD	Pre-diabetic; Obese (N=40) Mean ± SD	p-value
HbA1c (%)	5.28±.37	6.03±0.96	6.15±0.20	<0.001
Age (years)	39.03±5.72	38.39±6.31	39±5.20	0.78
FBS (mg/dl)	86.83±7.27	117.05±4.04	118.51±4.36	<0.001
SBP	123.40±7.41	131.80±8.06	126.41±7.77	<0.001
DBP	81.10±4.90	92.46±12.86	84.10±6.37	<0.001
Fasting insulin resistance	1.49±0.35	3.53±1.12	5.38±0.93	<0.001
PPBS (mg/dl)	93.76±12.5	152.64±9.32	167.26±14.33	<0.001
BMI (Kg/m^2)	23.80±2.08	24.15±1.63	26.13±0.54	<0.001
Fasting insulin (µIU/ml)	7.01±1.53	12.26±3.83	18.42±3.30	<0.001

Based on socio-demographic characteristics and biochemical parameters among study subjects. Socio-demographic parameters such as BMI, Systolic BP and diastolic BP showed a statistical significance with a p value <0.05. Whereas, age did not show any statistical significance. FBS, PPBS, HbA1c, Fasting insulin and Fasting insulin resistance also show statistical significance.

Table 4.2 Distribution of adipokines level and A/L ratio

Parameters	Control	Prediabetic-Non obese	Prediabetic-obese	p-value
Adiponectin (µg/ml)	13.17±2.8	7.49±2.34	7.26±1.94	<0.001
Leptin (ng/ml)	12.28±2.49	9.8±2.52	13.6±2.68	<0.001
Resistin (ng/ml)	13.1±4.06	16.59±3.86	17.60±3.60	<0.001
A/L ratio	1.15±0.37	0.79±0.40	0.56±0.22	<0.001

It was found that the serum adiponectin levels decreased significantly in obese prediabetes when compared with non-obese prediabetes and control subjects. Serum leptin level showed an elevated significance in obese prediabetes. In addition to that, it was observed that, serum resistin levels also elevated significantly among obese prediabetes when compared to non-obese prediabetes and control subjects. Whereas, significantly decreased A/L ratio was observed in obese prediabetes subjects was also observed (Table 4.2).

Table 4.3 Correlation between adiponectin level and fasting insulin resistance in study subjects

Variable	Control (N=150)		Pre-diabetic; Non-obese (N=110)		Pre-diabetic; Obese (N=40)	
Fasting insulin resistance	r	p-value	R	p-value	R	p-value
	0.148	0.143	0.031	0.119	0.471	0.812

In the case of level of adiponectin and fasting insulin resistance, there observed a weak positive association along with no statistically significance. In addition to that, the p value of prediabetic obese, non-obese and control was 0.812, 0.119 and 0.143 respectively (Table 4.3 and Fig. 4.2).
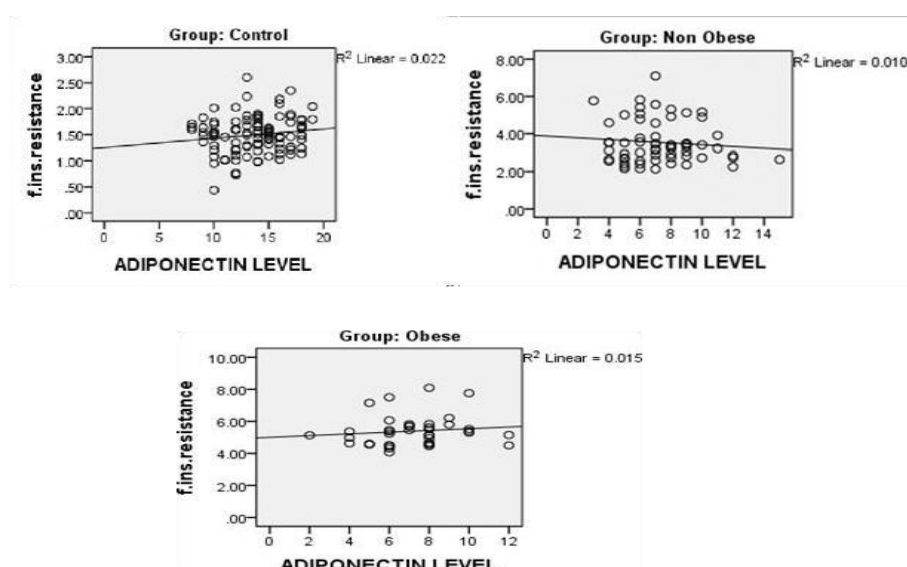
Fig. 4.2. Correlation between adiponectin level and fasting insulin resistance among study subjects

Table 4.4 Correlation between leptin level and fasting insulin resistance in study subjects

Variable	Control (N=150)		Pre-diabetic; Non-obese (N=110)		Pre-diabetic; Obese (N=40)	
	R	p-value	R	p-value	R	p-value
Fasting insulin resistance	0.173	0.182	0.275	0.091	0.105	0.299
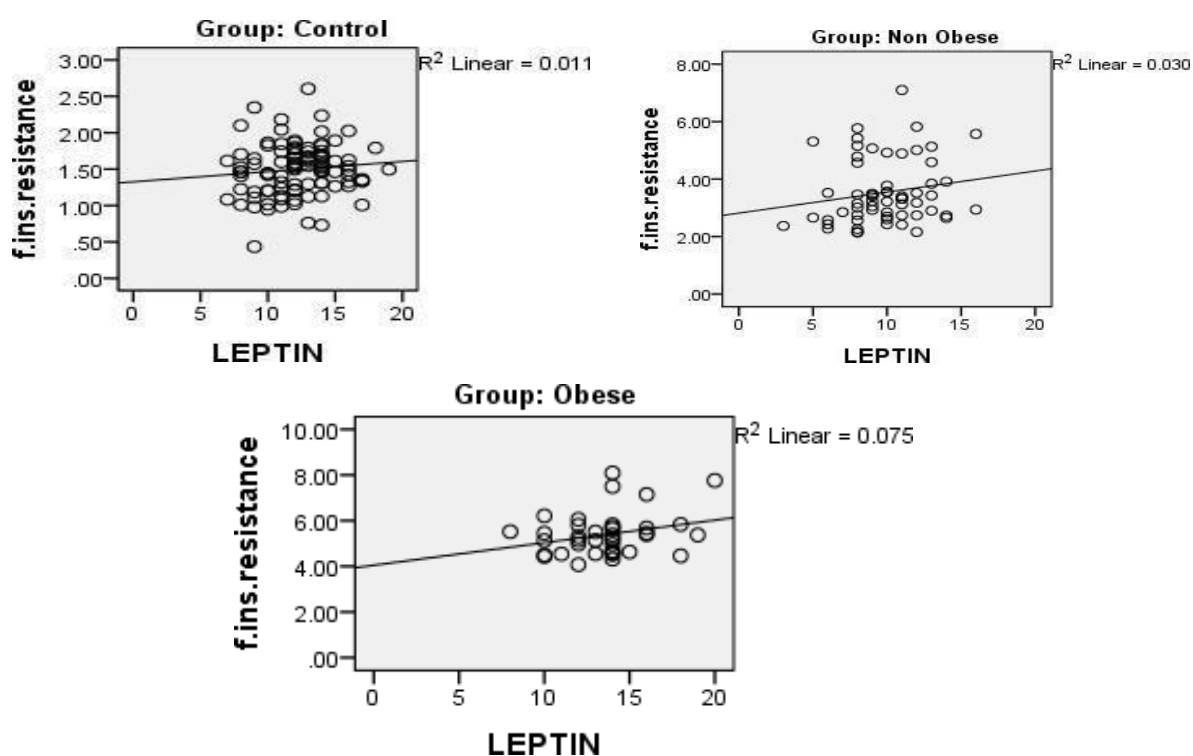
Fig. 4.3. Correlation between leptin level and fasting insulin resistance among study subjects

A weak positive correlation was observed in leptin level and fasting insulin resistance. Furthermore, an intermediate positive correlation among control group (p value 0.182). These results were statistically not significant (Table 4.4 and Fig. 4.3).

Table 4.5 Correlation between resistin level and fasting insulin resistance in study subjects

Variable	Control (N=150)		Pre-diabetic; Non-obese (N=110)		Pre-diabetic; Obese (N=40)	
	r	p-value	R	p-value	R	p-value
Fasting insulin resistance	0.021	0.833	- 0.179	0.167	-0.044	0.792
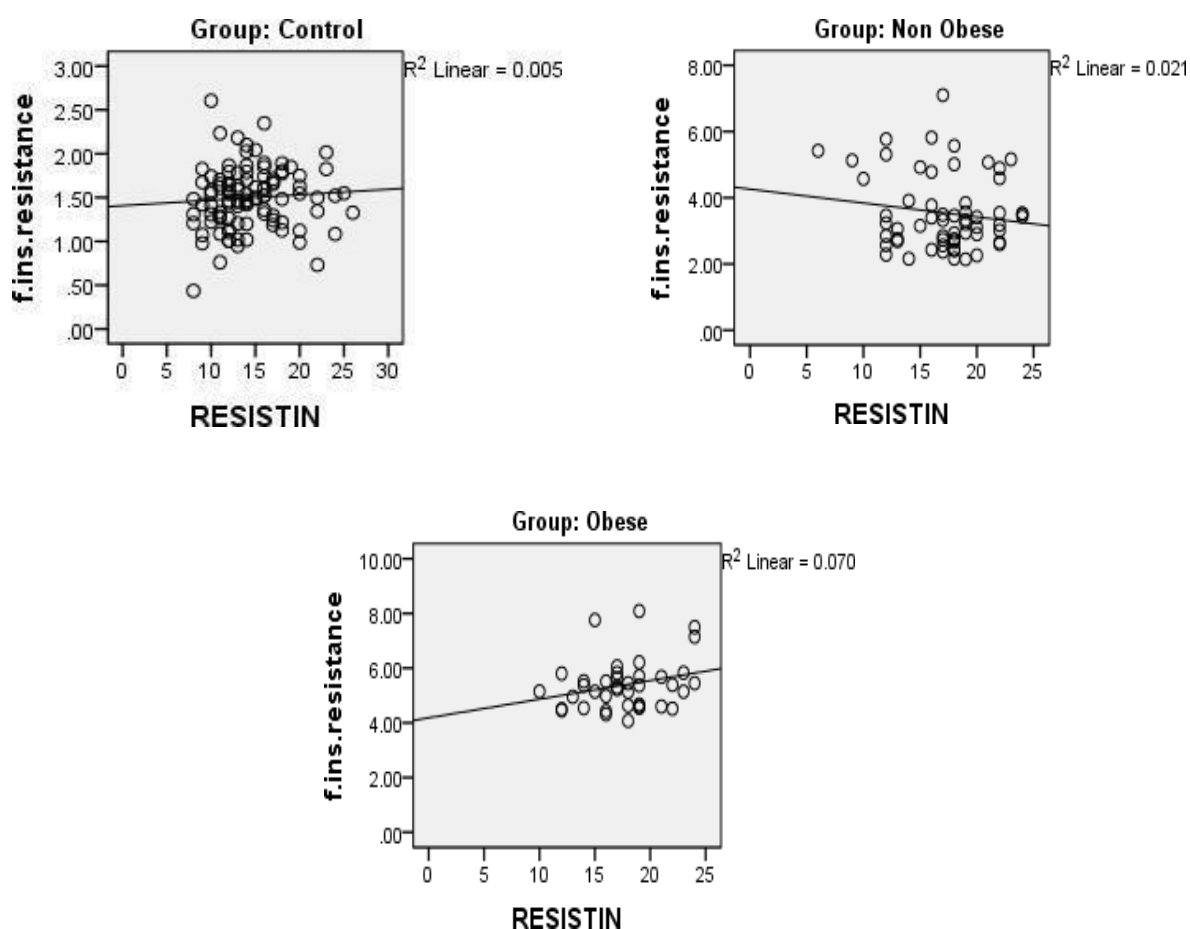
Fig. 4.4. Correlation between resistin level and fasting insulin resistance in study subjects

It was observed that, resistin level with fasting insulin resistance a weak positive correlation among control subjects and an intermediate positive correlation among obese subjects. Whereas, a negative weak correlation in non-obese group (Table 4.5 and Fig. 4.4).

The observed mean fasting insulin resistance among was prediabetes subjects with obesity was 5.38 ± 0.93 and for non-obese and control it was 3.53 ± 1.12 and 1.49 ± 0.35

respectively. Moreover, a statistical significance with p value $p < 0.001$ was observed (Table 4.1).

Adiponectin concentration was decreased significantly among obese subjects when compared to the rest reported as non-obese and along with the control subjects.

However, it was observed that, comparatively a higher level of serum leptin was found in obese group than the rest. A positive weak correlation was observed between leptin level and fasting insulin resistance whereas, a positive correlation was observed in control group.

The observed resistin concentration of obese, non-obese and control subjects were 17.60 ± 3.60, 16.59 ± 3.93 and 13.1 ± 4.06 respectively. In addition to that, a statistically significant difference with p value < 0.001 was also observed (Table 4.2). A positive weak correlation was found between resistin level with fasting insulin resistance.

Significantly decreased adiponectin-leptin ratio among obese prediabetes group was observed when compared to the rest ($p < 0.001$). A negative weak correlation of adiponectin-leptin ratio and fasting insulin resistance was also observed.

Table 4.6: Range, Mean, standard deviation and median of age

Group	Frequency	Range (age)	Mean	SD	Median
Control group	150	30-49	39.64	5.67	39.0
Prediabetic-non obese	110	30-49	39.03	5.90	38.0
Prediabetic -obese	40	31-49	39.08	5.16	38.0
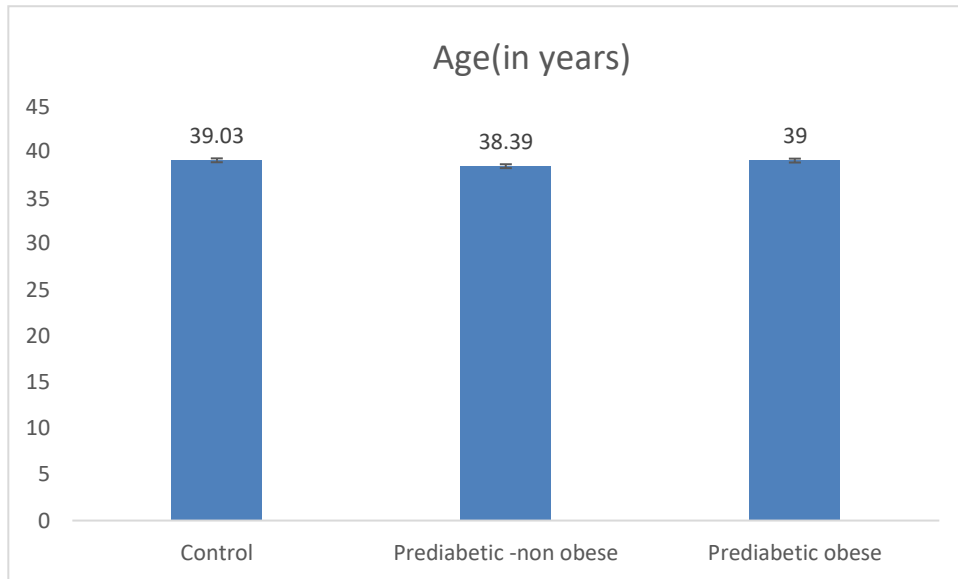
Fig:4.5 Distribution of Age according to study subjects

According to age the mean and median of study was evaluated and illustrated in the table no.4.6. Pre-diabetic-obese and non-obese showed a mean of 39.03 ± 5.90 and 39.08 ± 5.16 respectively. The observed mean of control group was 39.64 ± 5.67.

Table 4.7: Frequency and percentage distribution of samples according to gender

Gender	Control group		Pre- diabetic non – obese		Pre-diabetic-obese	
	F	%	f	%	f	%
Male	72	48.0	46	41.8	24	60.0
Female	78	52.0	64	58.2	16	40.0
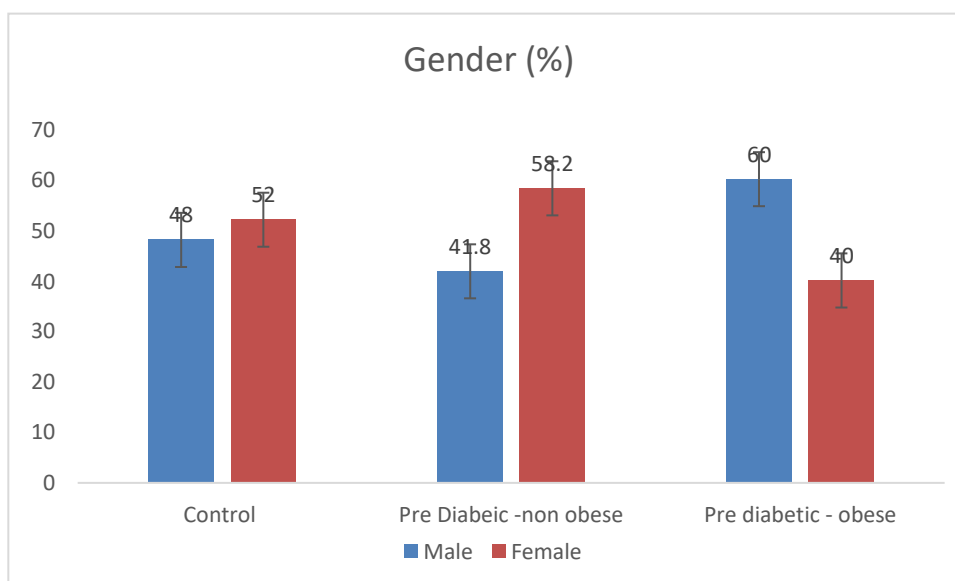
Fig: 4.6: Distribution of Gender according to study subjects

The distribution of study subjects according to gender was illustrated in the table no.4.7. In the current study 48% (f=72) were male control subjects and the remaining 52% (f=78) were female control subjects. Moreover, 41.8% were male Pre-diabetic non obese and 58.2% were female Pre-diabetic non obese. In case of, Pre-diabetic-obese group 60% were male and 40% were female.

Table 4.8: Range, Mean, standard deviation and median of BMI

Group	Frequency	Range	Mean	SD	Median
Control group	150	16.53-30.85	23.80	1.88	23.87
Prediabetic-non obese	110	20.13-30.48	24.15	1.80	24.20
Prediabetic -obese	40	24.97-28.13	26.13	0.56	26.12
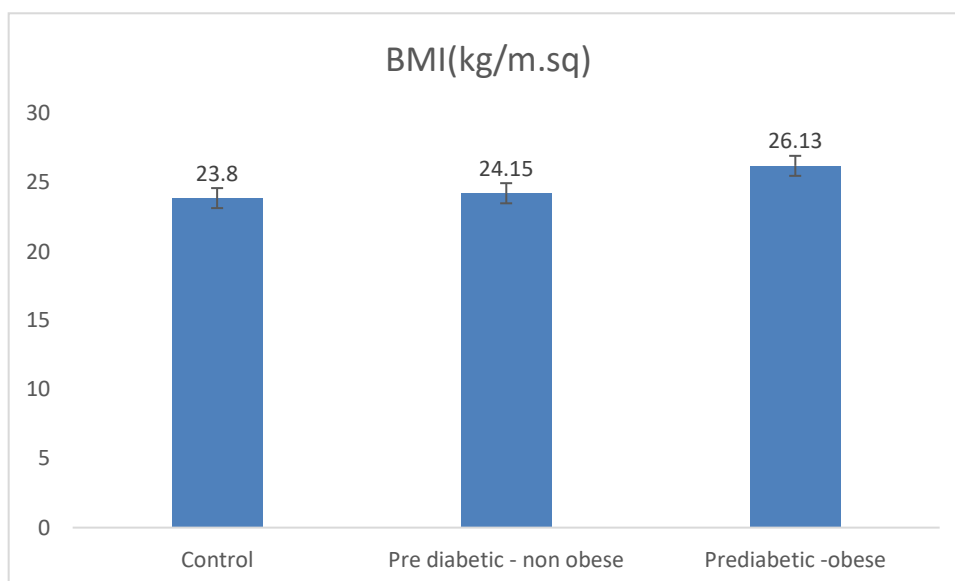
Fig: 4.7.: Distribution of BMI according to study subjects

Range and mean standard deviation based on BMI was illustrated in table no.4.8. The observed mean value of BMI among control subjects, Prediabetic-non obese and Prediabetic -obese were 23.8 ± 1.88, 24.15 ± 1.80, 26.13 ± 0.56 respectively.

Table 4.9: Range, Mean, standard deviation and median of systolic blood pressure

Group	Frequency	Range	Mean	SD	Median
Control group	150	110-140	123.40	7.41	120.0
Prediabetic non-obese	110	120-140	131.80	8.06	130.0
Prediabetic obese	40	120-140	126.41	7.77	120.0
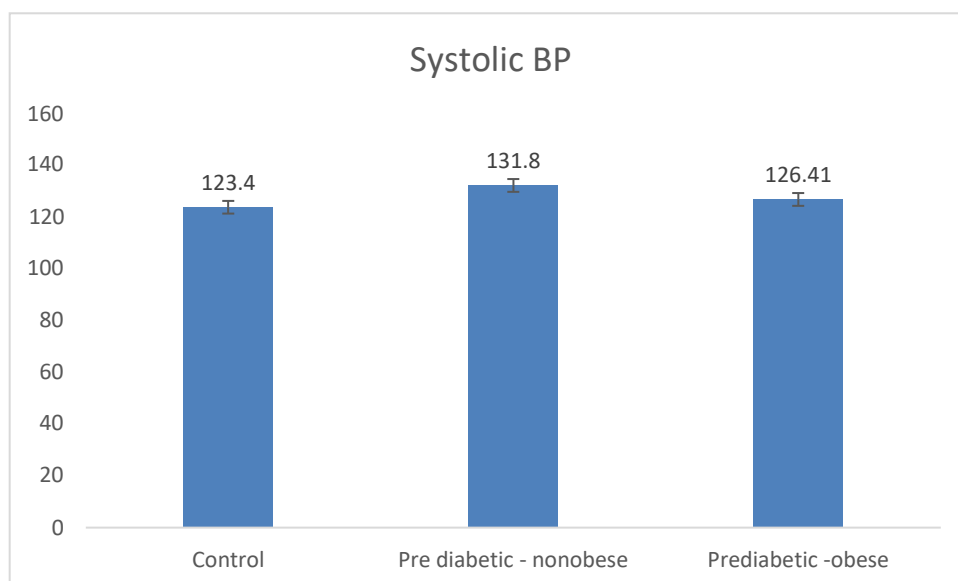
Fig: 4.8: Distribution of Systolic BP according to study subjects

Range, Mean, standard deviation and median according to systolic blood pressure was shown in table no.4.9. The mean Systolic BP of Control group 123.40 ± 7.41 was and for Prediabetic-non obese it was 131.80 ± 8.06 and for Prediabetic -obese was 126.41 ± 7.77.

Table 4.10: Range, Mean, standard deviation and median of diastolic blood pressure

Group	Frequency	Range	Mean	SD	Median
Control group	150	70-90	81.10	4.9	80.0
Prediabetic-non obese	110	70-110	92.46	12.86	90.0
Prediabetic -obese	40	70-100	84.10	6.37	80.0
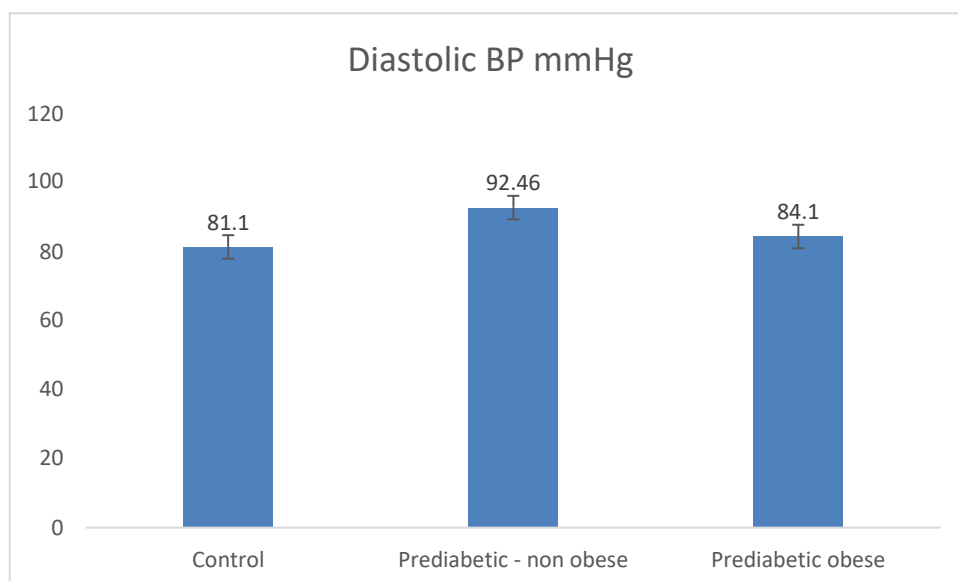
Fig: 4.9: Distribution of Diastolic BP according to study subjects

Control group showed a mean Diastolic BP of 81.1 ± 4.90. The observed mean Diastolic BP of Prediabetic-non obese and obese were 92.46 ± 12.86 and 84.10 ± 6.367 respectively.

Table 4.11: Range, Mean, standard deviation and median of Fasting Insulin resistance

Group	Frequency	Range	Mean	SD	Median
Control group	150	0.44-2.60	1.49	0.35	1.30
Prediabetic-Non obese	110	1.56-5.82	3.53	1.12	2.94
Prediabetic obese	40	0.99-5.80	5.38	0.93	5.19
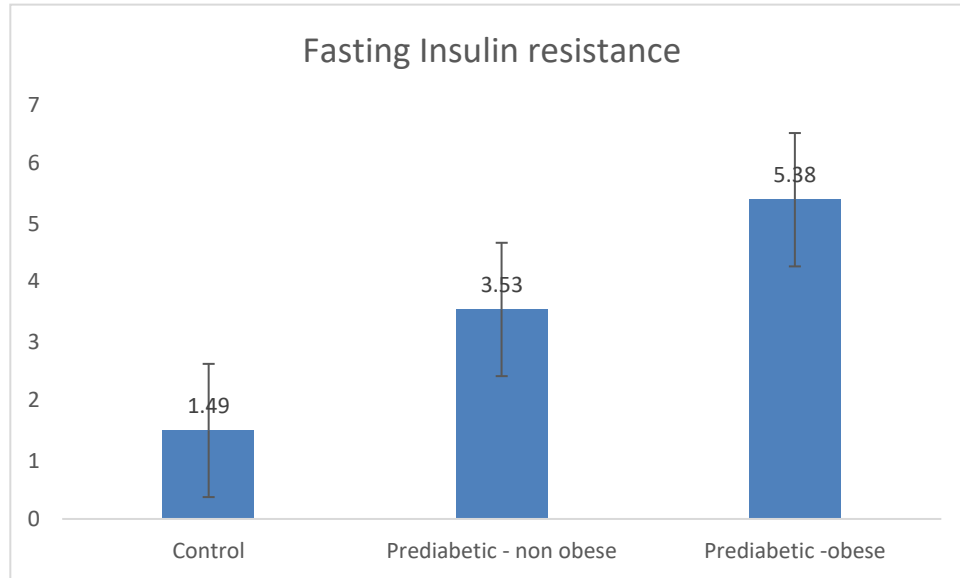
Fig: 4.10: Distribution of Fasting Insulin resistance according to study subjects

Range, Mean, standard deviation and median of Fasting Insulin resistance was illustrated in table no.4.11. Control group showed a mean Fasting Insulin resistance of 1.49 ± 0.35, Prediabetic-non obese showed a mean Fasting Insulin resistance 3.53± 1.12 and for Prediabetic-obese it was 5.38 ± 0.93.

Table 4.12: Range, Mean, standard deviation and median of Fasting Insulin

Group	Frequency	Range	Mean	SD	Median
Control group	150	2.10-11.10	7.01	1.53	7.20
Prediabetic-non obese	110	5.50-19.00	12.26	3.83	11.35
Prediabetic-obese	40	3.50-18.80	18.42	3.30	17.8
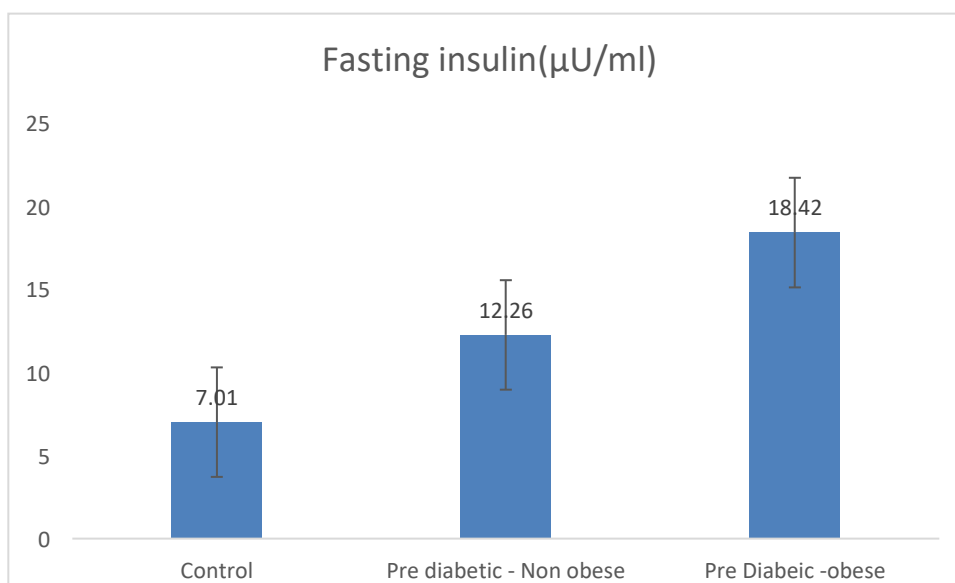
Fig: 4.11: Distribution of Fasting Insulin according to study subjects

The mean Fasting Insulin of control group was 7.01 ± 1.53, for Prediabetic-non obese it was 12.26 ± 3.83 and for Prediabetic -obese it was 18.42 ± 3.30.

Table 4.13: Range, Mean, standard deviation and median of fasting blood sugar

Group	Frequency	Range	Mean	SD	Median
Control group	150	70-104	86.83	7.27	87.0
Prediabetic-non obese	110	111-125	117.05	4.04	116
Prediabetic obese	40	111-128	118.51	4.36	117
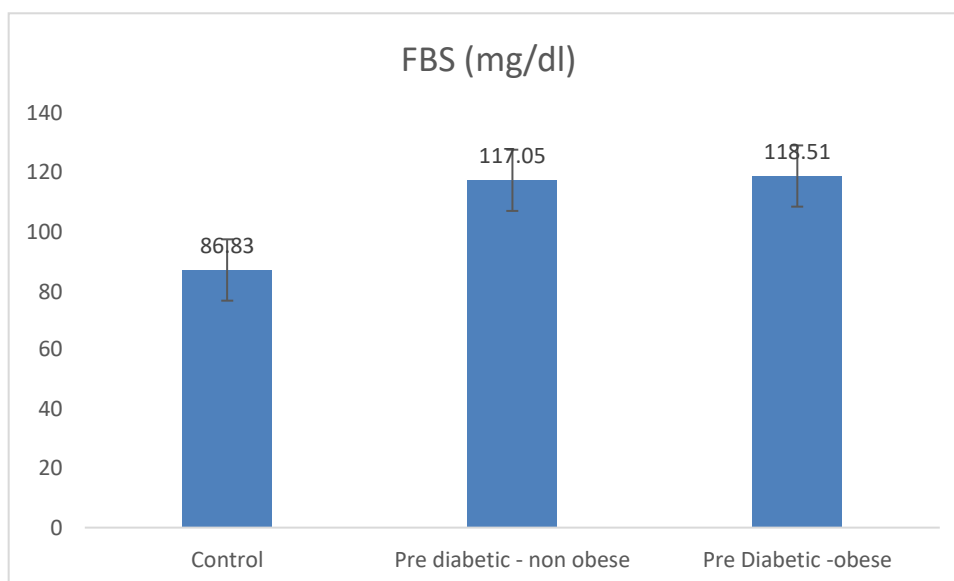
Fig: 4.12: Distribution of FBS according to study subjects

Range, Mean, standard deviation and median of fasting blood sugar was mentioned in table no.4.13. The observed mean FBS of control was 86.83 ± 7.27. The observed mean FBS of Prediabetic-non obese and Prediabetic-obese was 117.05±4.04 and 118.5 ± 4.36 respectively.

Table 4.14: Range, Mean, standard deviation and median of Post Prandial Blood Sugar

Group	Frequency	Range	Mean	SD	Median
Control group	150	72-118	93.76	12.5	95.0
Prediabetic-nonobese	110	129-185	152.64	9.32	152.0
Prediabetic-obese	40	140-190	167.26	14.33	168.0
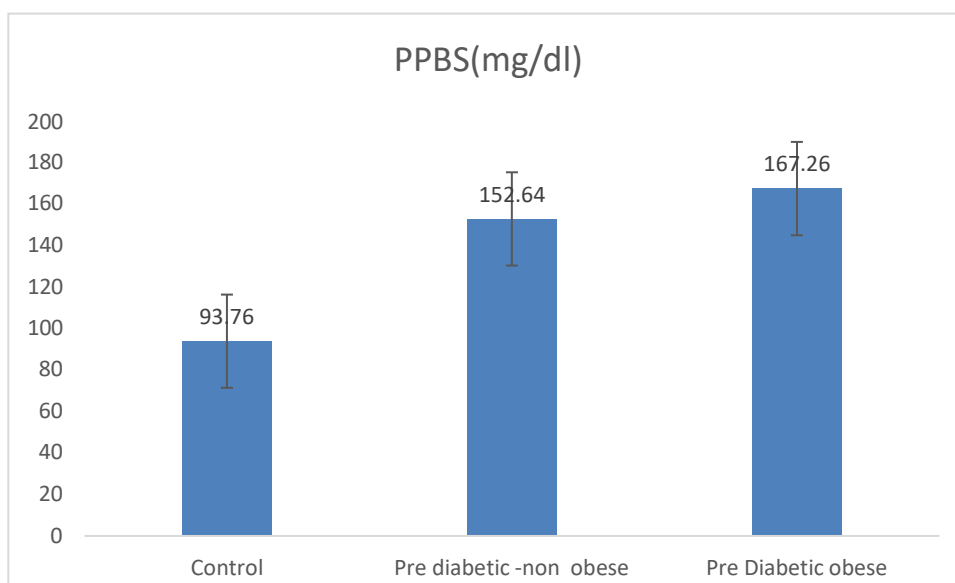
Fig: 4.13: Distribution of PPBS according to study subjects

Mean of Post Prandial Blood Sugar among study subjects was shown in table no.4.14. Control group showed a mean PPBS of 93.76± 12.5, whereas, the observed mean PPBS of Prediabetic-non obese was 152.64±9.32 and for Prediabetic -obese it was 167.26±14.33.

Table 4.15: Range, Mean, standard deviation and median of HBA1c

Group	Frequency	Range	Mean	SD	Median
Control group	150	4.30-5.90	5.28	0.37	5.30
Prediabetic-non obese	110	5.70-6.50	6.03	0.96	6.10
Prediabetic -obese	40	5.70-6.40	6.15	0.20	6.00
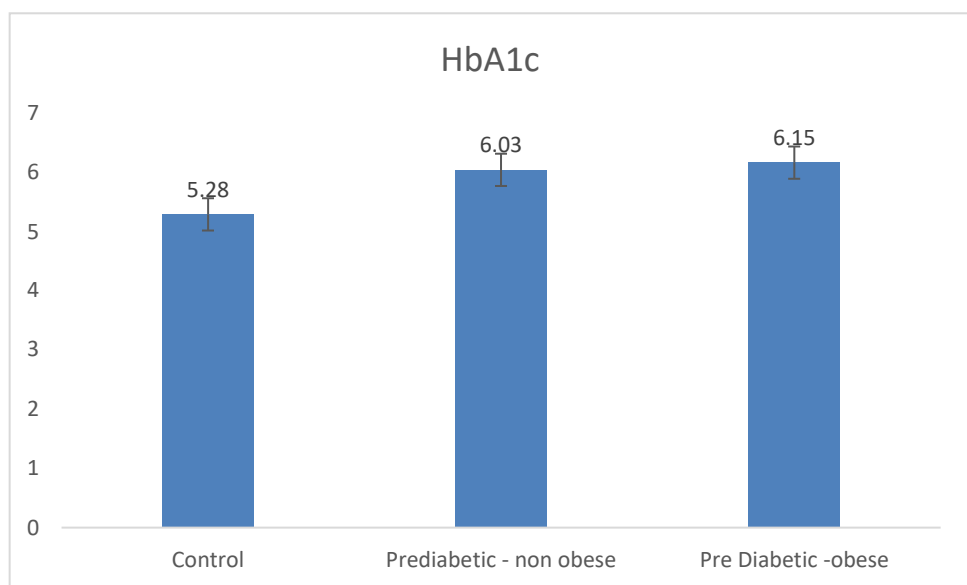
Fig: 4.14: Distribution of HbA1c according to study subjects

Control group showed a mean HbA1c of 5.28±0.37. Prediabetic nonobese showed a mean HbA1c of 6.03±0.96. The observed mean HbA1c of Prediabetic-obese was 6.15±0.20.

Table 4.16: Range, Mean, standard deviation and median of Adiponectin level

Group	Frequency	Range	Mean	SD	Median
Control group	150	5.50-18.90	13.17	2.80	13.5
Prediabetic-non obese	110	2.80-15.20	7.49	2.34	7.2
Prediabetic -obese	40	2.50-12.50	7.26	1.94	7.4
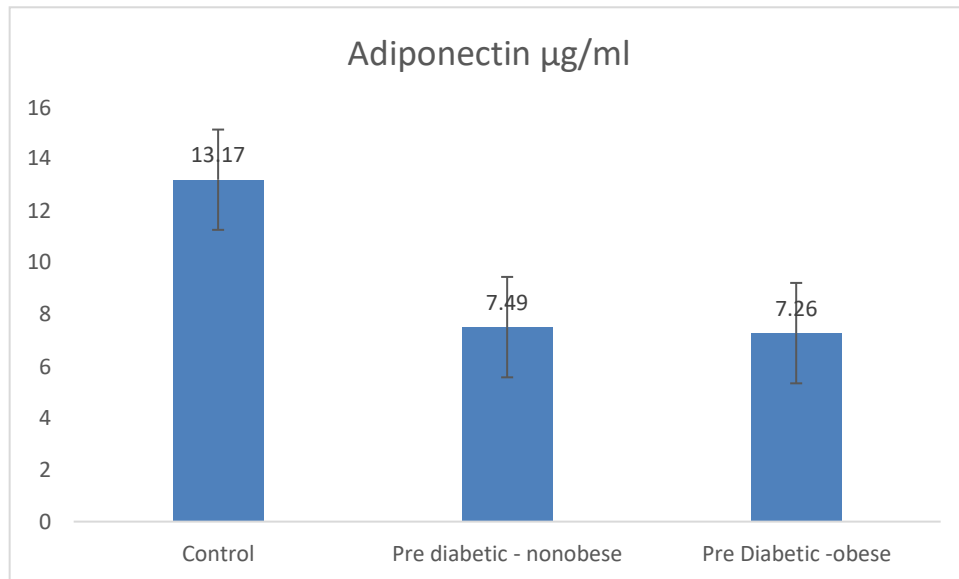
Fig: 4.15: Distribution of Adiponectin according to study subjects

Control group showed a mean Adiponectin level of 13.17±2.80 The observed mean Adiponectin level of Prediabetic-non obese was 7.49±2.34 and for Prediabetic-obese it was 7.26±1.94.

Table 4.17: Range, Mean, standard deviation and median of Leptin level

Group	Frequency	Range	Mean	SD	Median
Control group	150	7.0-18.80	12.28	2.49	12.15
Prediabetic -non obese	40	8.50-19.60	9.8	2.52	9.9
Prediabetic- obese	110	3.12-15.50	13.6	2.68	13.5
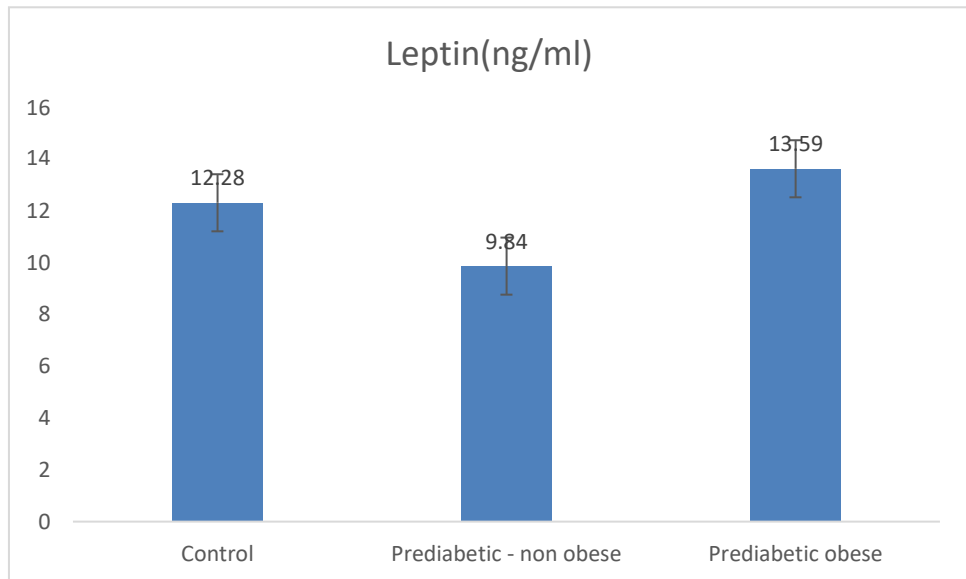
Fig: 4.16: Distribution of Leptin according to study subjects

Range, Mean, standard deviation and median of Leptin level was denoted in table no.4.17. The observed mean Leptin of control group, Prediabetic-obese and Prediabetic-non obese were 12.28±2.49, 13.6±2.68 and 9.8±2.52 respectively.

Table 4.18: Range, Mean, standard deviation and median of Resistin level

Group	Frequency	Range	Mean	SD	Median
Control group	150	6.00-26.50	13.1	4.06	13.0
Prediabetic-non obese	110	6.50-24.20	16.59	3.86	17.0
Prediabetic-obese	40	10.0-24.0	17.60	3.59	17.5
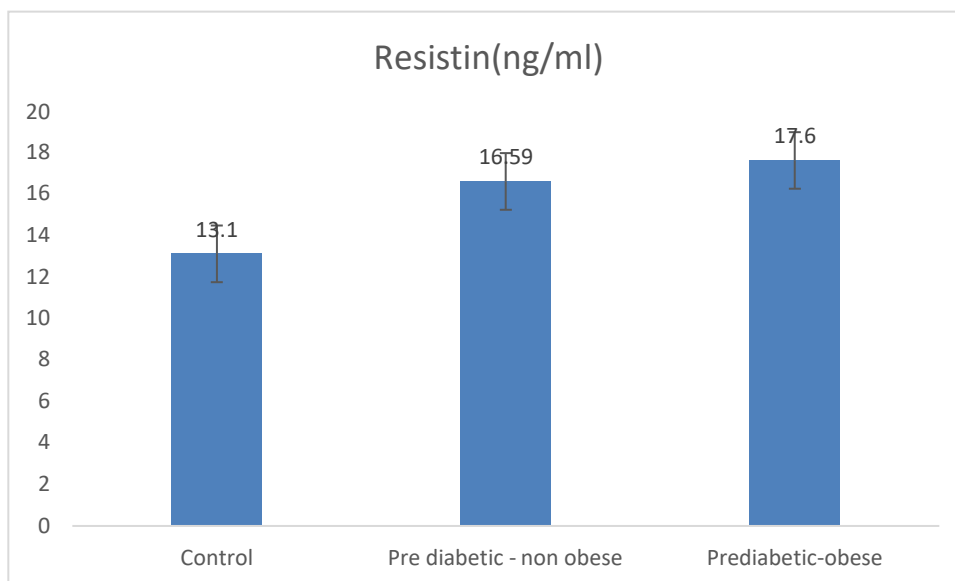
Fig: 4.17: Distribution of Resistin according to study subjects

Distribution of mean Resistin level among study subjects was illustrated in the above table no 4.18. It was observed the mean Resistin of control group was13.17±4.06, and for Pre-diabetic-non obese it was 16.59±3.86 and for Pre-diabetic -obese it was 17.60±3.6.

Table 4.19: Frequency and percentage distribution of samples according to Adiponectin SNP

Adiponectin SNP	Control group		Pre-diabetic-obese		Pre-diabetic -non obese	
	F	%	f	%	f	%
CC	84	56.0	23	20.9	13	32.5
CG	34	22.7	41	37.3	14	35.0
GG	32	21.3	46	41.8	13	32.5
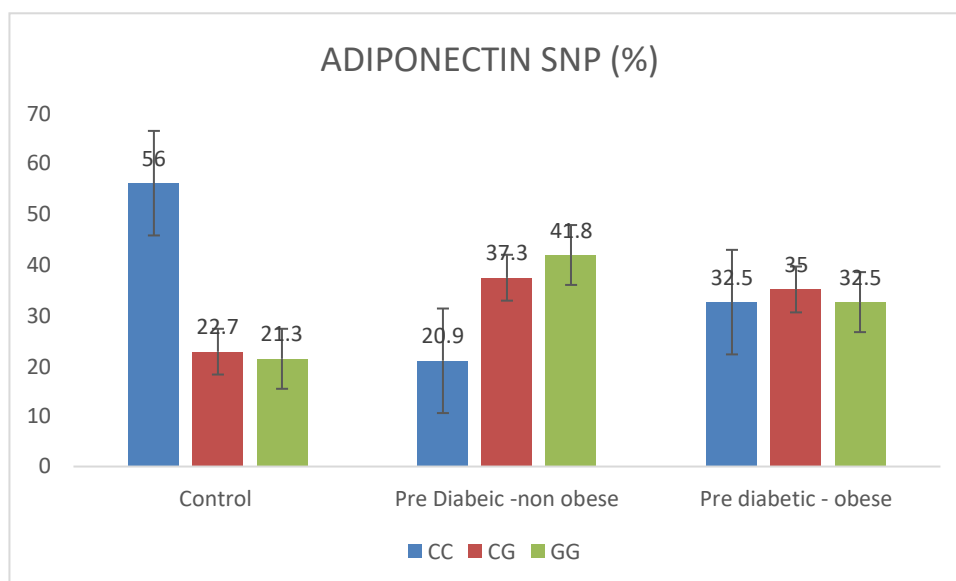
Fig: 4.18: Frequency and percentage distribution of samples according to Adiponectin SNP

Frequency and percentage distribution of samples according to Adiponectin SNP was explained in table no.4.19.

Table 4.20: Frequency and percentage distribution of samples according to Leptin SNP

Leptin SNP	Control group		Pre-diabetic-obese		Pre-diabetic -non obese	
	f	**%**	**f**	**%**	**f**	**%**
AA	27	18.0	20	18.2	11	27.5
GA	60	40.0	66	60.0	22	55.0
GG	63	42.0	24	21.8	7	17.5

68
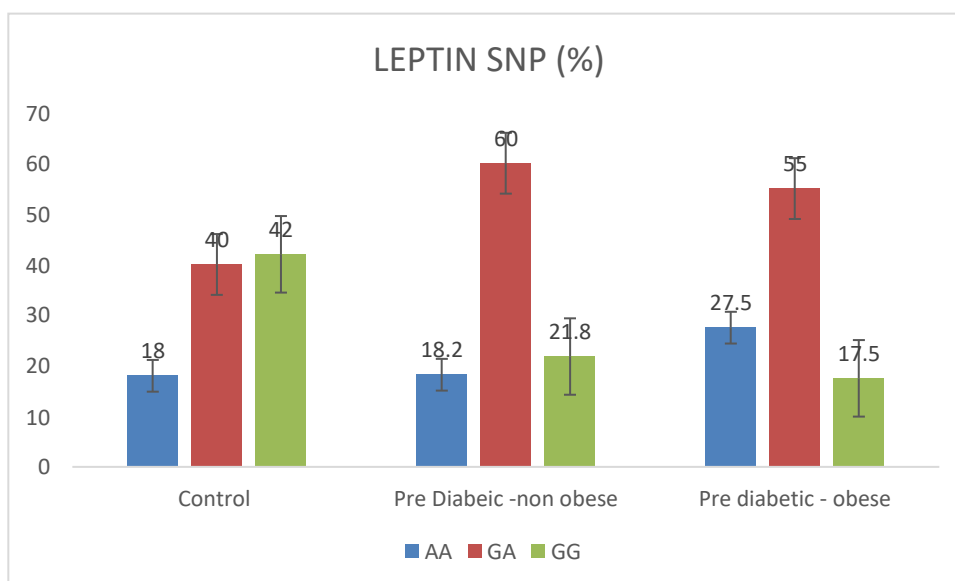
Fig: 4.19: Distribution of samples according to Leptin SNP

Frequency and percentage distribution of samples according to Leptin SNP was shown in table no.4.20.

Table 4.21: Frequency and percentage distribution of samples according to Resistin SNP

Resistin SNP	Control group		Pre-diabetic-obese		Pre-diabetic -non obese	
	F	**%**	**F**	**%**	**f**	**%**
CC	60	40.0	43	39.1	12	30.0
CG	59	39.3	43	39.1	22	55.0
GG	31	20.7	24	21.8	6	15.0

69
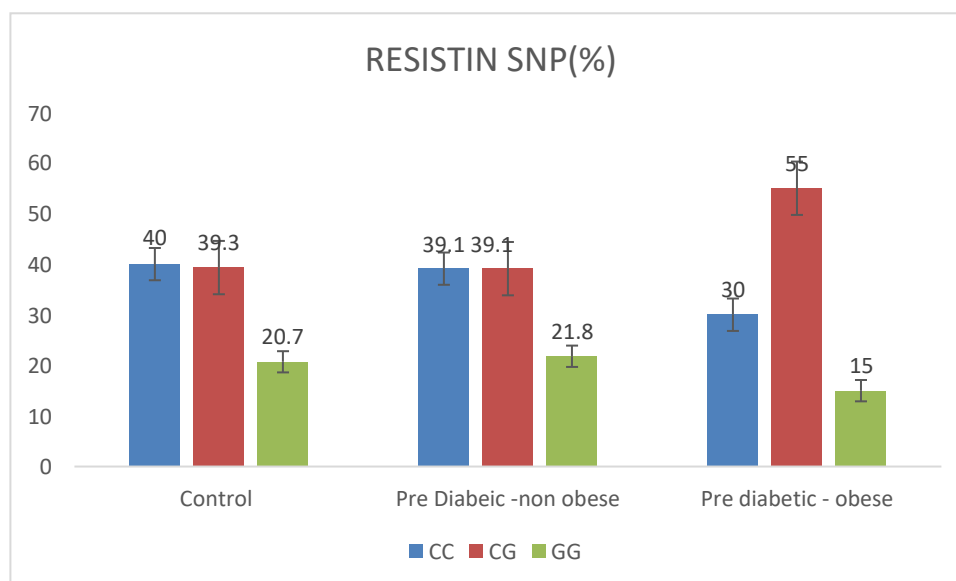
Fig: 4.20: Distribution of samples according to Resistin SNP

Frequency and percentage distribution of samples according to Resistin SNP among study subjects was shown in illustrated table no.4.21.

Table 4.22: Association of Adiponectin level with Adiponectin Single Nucleotide Polymorphisms in control group

Adiponectin SNP	Adiponectin level µg/dl		Total	χ^2 value	p value
	Median (≤13.50)	Median (>13.50)			
CC	44	40	84	1.549	0.461 (NS)
CG	15	19	34		
GG	19	13	32		

NS- Not significant

Data presented in table 4.22 showed that, there was no significant association between Adiponectin level with Adiponectin Single Nucleotide Polymorphisms in control group (χ^2 = 1.549, p>0.05).

70

Table 4.23: Association of Leptin level with Leptin Single Nucleotide Polymorphisms in control group

Leptin SNP	Leptin level ng/dl		Total	χ^2 value	p value
	Median ($\leq$12.15)	Median (>12.15)			
AA	15	12	27		
GA	31	29	60	0.797	0.671 (NS)
GG	29	34	63		

NS- Not significant

In table 4.23 showed that there was no any significant association between Leptin level with Leptin SNP in control group (χ^2 = 0.797, p>0.05).

Table 4.24: Association of Resistin level with Resistin Single Nucleotide Polymorphisms in control group

Resistin SNP	Resistin level ng/dl		Total	χ^2 value	p value
	Median ($\leq$13.00)	Median (>13.00)			
CC	42	18	60		
CG	32	27	59	5.004	0.082 (NS)
GG	15	16	31		

NS- Not significant

Data presented in table 4.24 showed that there was no any significant association between Resistin level with Resistin Single Nucleotide Polymorphisms in control group (χ^2 = 5.004, p>0.05).

Table 4.25: Association of Adiponectin level with Adiponectin Single Nucleotide Polymorphisms in non-obese prediabetic group

Adiponectin SNP	Adiponectin level		Total	χ^2 value	p value
	Median (≤7.20)	Median (>7.20)			
CC	3	20	23		
CG	25	16	41	16.684	<0.001**
GG	28	18	46		

** Significant at 0.001 level

Data presented in table 4.25 showed that there was a significant association between Adiponectin level with Adiponectin SNP in **non-obese pre-diabetic group** ($\chi^2 =$ 16.684, p<0.001).

Table 4.26: Association of Leptin level with Leptin Single Nucleotide Polymorphisms in non-obese pre-diabetic group

N=110

Leptin SNP	Leptin level		Total	χ^2 value	p value
	Median (≤10.40)	Median (>10.40)			
AA	7	13	20		
GA	32	34	66	7.725	0.021*
GG	18	6	24		

*Significant at 0.05 level

Data presented in table 4.26 showed that there was significant association between leptin level with leptin SNP in **non-obese pre-diabetic group** ($\chi^2 =$ 7.725, p>0.05).

Table 4.27: Association of Resistin level with Resistin Single Nucleotide Polymorphisms in non-obese pre-diabetic group (N=110)

Resistin SNP	Resistin level		Total	χ^2 value	p value
	Median (≤17.00)	Median (>17.00)			
CC	24	19	43	0.813	0.666 (NS)
CG	20	23	43		
GG	13	11	24		

NS- Not significant

In table 4.27 showed that there was no any significant association between resistin level with resistin SNP in **non-obese pre-diabetic group** (χ^2 = 0.813, p>0.05).

Table 4.28: Association of Adiponectin level with Adiponectin Single Nucleotide Polymorphisms in obese prediabetic group (N=40)

Adiponectin SNP	Adiponectin level		Total	χ^2 value	p value
	Median (≤7.20)	Median (>7.20)			
CC	2	11	13	12.462	0.002*
CG	7	7	14		
GG	11	2	13		

*Significant at 0.01 level

Data presented in table 4.28 showed that there was significant association between Adiponectin level with Adiponectin SNP in **obese prediabetic group** (χ^2 = 12.462, p>0.05).

Table 4.29: Association of Leptin level with Leptin Single Nucleotide Polymorphisms in obese prediabetic group (N=40)

Leptin SNP	Leptin level		Total	χ^2 value	p value
	Median (≤10.40)	Median (>10.40)			
AA	3	9	12	6.973	0.031*
GA	14	8	22		
GG	5	1	6		

*Significant at 0.05 level

Data presented in table 4.29 showed that there was significant association between leptin level with leptin SNP in **obese prediabetic group** ($\chi^2 = 6.973$, p>0.05).

Table 4.30: Association of Resistin level with Resistin Single Nucleotide Polymorphisms in obese prediabetic group (N=40)

Resistin SNP	Resistin level		Total	χ^2 value	p value
	Median (≤17.00)	Median (>17.00)			
CC	7	5	12	1.000	0.607 (NS)
CG	11	11	22		
GG	2	4	6		

NS- Not significant

Data presented in table 4.30 showed that there was no any significant association between resistin level with resistin SNP in **obese prediabetic group** ($\chi^2 = 1.000$).

Table 4.31: Multiple regression on Factors determining of insulin resistance (N=300)

Factors	B	SE	β	95% CI of B	t value	p value
Constant	.011	.130		-0.245- 0.266	0.082	0.935
Age in years	.003	.002	.014	-0.001- 0.007	1.300	0.195
F. Insulin	.298	.004	.904	0.290-0.306	74.710	<0.001
Adiponectin level	-.042	.004	-.141	0.049-0.034	11.270	<0.001
Leptin level	-.007	.005	-.017	-0.016- 0.002	-1.500	0.135
Resistin level	.006	.003	.022	0.000- 0.012	1.901	0.058

$R^2 = 0.966$, F (5, 294) = 1684.100, p <0.001

Multiple linear regression was performed to determine the factors behind the fasting insulin resistance table no 4.31. Overall regression model was statistically significant ($R^2 = 0.966$, F (5, 294) = 1684.100, p <0.001). The predictor 'F. Insulin' (p<0.001), was statistically significant with the fasting insulin resistance.

Table 4.32: Multiple regression on Factors determining of insulin resistance in control group (N=150)

Factors	B	SE	β	95% CI of B	t value	p value
Constant	-.067	.109		-0.282- 0.149	0.611	0.542
Age in years	.000	.002	-0.002	-0.004- 0.003	0.087	0.931
F. Insulin	.216	.006	0.944	0.204-0.227	36.003	<0.001
Adiponectin level	.003	.004	0.021	-0.004-0.010	0.839	0.403
Leptin level	-.002	.004	-0.010	-0.010- 0.007	0.379	0.705
Resistin level	.003	.003	0.033	0.002- 0.008	1.257	0.211

$R^2 = 0.915$, F (5, 144) = 309.550, p <0.001

Multiple linear regression was performed to determine the factors behind the fasting insulin resistance among samples in control group table no 4.32. Overall regression model was statistically significant at 0.001 level of significance ($R^2 = 0.915$, F (5, 144) = 309.550, p <0.001). The predictor 'F. Insulin' (p<0.001), was statistically significant with the f. insulin resistance.

Table 4.33: Multiple regression on Factors determining of insulin resistance in prediabetes non obese group

Factors	B	SE	β	95% CI of B	t value	p value
Constant	-0.021	0.117		-0.253- 0.210	-0.018	0.854
Age in years	0.002	0.002	0.015	-0.002- 0.006	1.087	0.279
F. Insulin	0.281	0.004	0.989	0.273- 0.289	71.852	<0.001
Adiponectin level	0.000	0.005	0.000	-0.010 -0.010	0.014	0.989
Leptin level	0.004	0.004	0.012	-0.005- 0.013	0.884	0.379
Resistin level	-0.001	0.003	-0.003	0.007- 0.005	-0.195	0.845

$R^2 = 0.981$, F (5, 104) = 1054.646, p <0.001

Multiple linear regression was performed to determine the factors behind the f. insulin resistance among samples in prediabetic non obese group table no4.33. Overall regression model was statistically significant at 0.001 level of significance ($R^2 = 0.981$, F (5, 104) = 1054.646, p <0.001). The predictor 'F. Insulin' (p<0.001), was statistically significant with the f. insulin resistance.

Table 4.34: **Multiple regression on factors determining of insulin resistance in prediabetic obese group. (N=40)**

Factors	B	SE	β	95% CI of B	t value	p value
Constant	0.148	0.263		-0.386- 0.682	0.563	.577
Age in years	-0.003	0.004	-0.014	-0.012- 0.005	-0.830	0.412
F. Insulin	0.290	0.005	0.998	0.279- 0.300	58.355	<0.001
Adiponectin level	0.007	0.011	0.010	-0.016 -0.029	0.610	0.546
Leptin level	0.004	0.008	0.007	-0.013- 0.020	0.444	0.660
Resistin level	-0.005	0.006	-0.014	0.017- 0.008	-0.785	0.438

$R^2 = 0.992$, F (5, 34) = 848.652, p <0.001

Multiple linear regression was performed to determine the factors behind the f. insulin resistance among samples in prediabetes obese group table no 4.34. Overall regression model was statistically significant at 0.001 level of significance ($R^2 = 0.992$, F (5, 34) = 848.652, p <0.001). The predictor 'F. Insulin' (p<0.001), was statistically significant with the f. insulin resistance.

Table No.4.35: Genotype and Allele association of Adiponectin polymorphism between prediabetic and controls

Polymorphism	Prediabetics N (%)	Controls N (%)	OR (95% CI)	p value
Co Dominant				
CC	36(24)	84(56)	1	-
CG	55(36.7)	34(22.7)	0.265(0.148,0.473)	<0.001
GG	59(39.3)	32(21.3)	0.232(0.130,0.416)	<0.001
Dominant				
CC	36(24)	84(56)	1	-
CG+GG	114(76)	66(44)	0.248(0.151,0.407)	<0.001

Polymorphism	Prediabetics N (%)	Controls N (%)	OR (95% CI)	p value
Recessive				
CG+CC	91(60.7)	118(78.7)	1	-
GG	59(39.3)	32(21.3)	0.418(0.251,0.696)	0.001
Alleles				
C	127(42.3)	202(67.3)	1	-
G	173(57.7)	98(32.7)	0.356(0.255-0.497)	<0.001

Table no.4.35., demonstrated the distribution and association of Genotype and Allele of Adiponectin polymorphism between pre diabetic and control group. The co-dominant, dominant, recessive and alleles found to have statistically significant association for cases as compared to controls.

Table No 4.36: Genotype and Allele association of Leptin polymorphism between prediabetics and controls

Polymorphism	Prediabetics n (%)	Controls n (%)	OR (95% CI)	p value
Co Dominant				
GG	31(20.7)	63(42)	1	-
GA	88(58.7)	60(40)	0.335(0.195,0.576)	<0.001
AA	31(20.7)	27(18)	0.429(0.219,0.839)	0.013
Dominant				
GG	31(20.7)	63(42)	1	-
GA+AA	119(79.3)	87(58)	0.36(0.216,0.6)	<0.001
Recessive				
GA+GG	119(79.3)	123(80)	1	-
AA	31(20.7)	27(18)	0.843(0.475,1.496)	0.004
Alleles				
G	150(50)	186(62)	1	-
A	150(50)	114(38)	0.613(0.443,0.848)	0.003

Table 4.36 shows, the association of co dominant, dominant, recessive genotype and allele between cases and controls were found to be statistically significant. As compared to the reference category of all genotypes and allele the prominent polymorphisms were less likely to occur for cases than controls.

Table No.4.37: Genotype and Allele association of resistin polymorphism between prediabetics and controls

Polymorphism	prediabetics n (%)	Controls n (%)	OR (95% CI)	p value
Co Dominant				
CC	55(36.7)	60(40)	1	-
CG	65(43.3)	59(39.3)	0.832(0.501-1.383)	0.478
GG	30(20)	31(20.7)	0.947(0.509-1.763)	0.864
Dominant				
CC	55(36.7)	60(40)	1	-
CG+GG	95(63.3)	90(60)	0.868(0.545-1.384)	0.553
Recessive				
CG+CC	120(80)	119(79.3)	1	-
GG	32(20)	31(20.7)	1.042(0.596-1.829)	0.886
Alleles				
C	175(58.3)	179(59.7)	1	-
G	125(41.7)	121(40.3)	0.946(0.683-1.31)	0.74

Table 4.37 shows that, none of the polymorphisms showed significant association with cases as compared to controls.

All the SNP markers at the ADIPOQ (rs266729), LEP (rs7799039) and RETN (rs1862513) loci were in Hardy-Weinberg Equilibrium. In statistical analysis, it showed that there was no any significant association between adiponectin level with adiponectin SNP in control group and there was a significant association between adiponectin level with adiponectin SNP in obese and non-obese prediabetes. In table no.4.35, genotype and allele frequencies of rs266729 promoter region ADIPOQ gene polymorphism was illustrated. In prediabetes group the observed frequency of CC genotype was of rs266729 was 36% and for CG it was 55% and for GG it was 59%.

Significant correlations between the homozygous CC and heterozygous CG genotypes and prediabetes were found. The recessive genotype CG+CC vs GG similarly showed a significant difference between prediabetes patient and healthy control (OR: 0.418, Cl:0.251-0.696 :P<0.001). In statistical analysis, it was found that there was a

significant connection between leptin level and leptin SNP in obese and non-obese prediabetes but not in the control group.

The genotype and allele frequencies of rs7799039 promoter region LEP gene polymorphism in prediabetes and healthy control are shown in table 4.36. The frequency of the GG, GA and AA genotype of rs7799039 were 20.7%, 58.7% and 20.7% in prediabetes case and 42%, 40%, 18% in healthy control respectively. The allele frequency of G and A were 50%,50% in prediabetes and 62%,38%in healthy control respectively. There were significant association of homozygous GG and heterozygous GA genotype with prediabetes (OR:0.335, Cl:0.195-0.576:P<0.001) and GA allele frequency of rs7799039 had significant association with prediabetes as compared to healthy controls (OR:0.613, Cl:0.443-0.848: P=0.003).it also analyzed the dominant genotype (GG vs GA+AA) and found significant difference between prediabetes case and healthy control (OR:0.36, Cl:0.216-0.6:P<0.001). Similarly, the recessive genotype GA+GG vs AA did show significant difference between prediabetes case and healthy control (OR:0.843, Cl:0.475-0.496 :P=0.004).

In statistical analysis, it showed that there was no any significant association between resistin level with resistin SNP in control group and there was no significant association between resistin level with resistin SNP in obese and non-obese prediabetes.

The genotype and allele frequencies of rs1862513 promoter region RETN gene polymorphism was shown in table 4.37.

In prediabetes cases, the frequency of the CC, CG, and GG genotypes was 36.7%, 43.3%, and 20%, respectively, while it was 40%, 39.3%, and 20.7% in healthy controls.

The homozygous CC and heterozygous CG genotypes did not significantly correlate with prediabetes. Similarly, CG allele frequency of rs1862513 had no significant association with prediabetes as compared to healthy controls.

The dominant genotype (CC vs CG+GG) and found no significant difference between prediabetes case and healthy control (OR:0.868, CI:0.545-1.384:P<0.55).

Similarly, the recessive genotype CG+CC vs GG did not show significant difference between prediabetes case and healthy control (OR:1.042, Cl:0.596-1.829 :P=0.89).

Isolated genomic DNA quantified by spectrophotometer
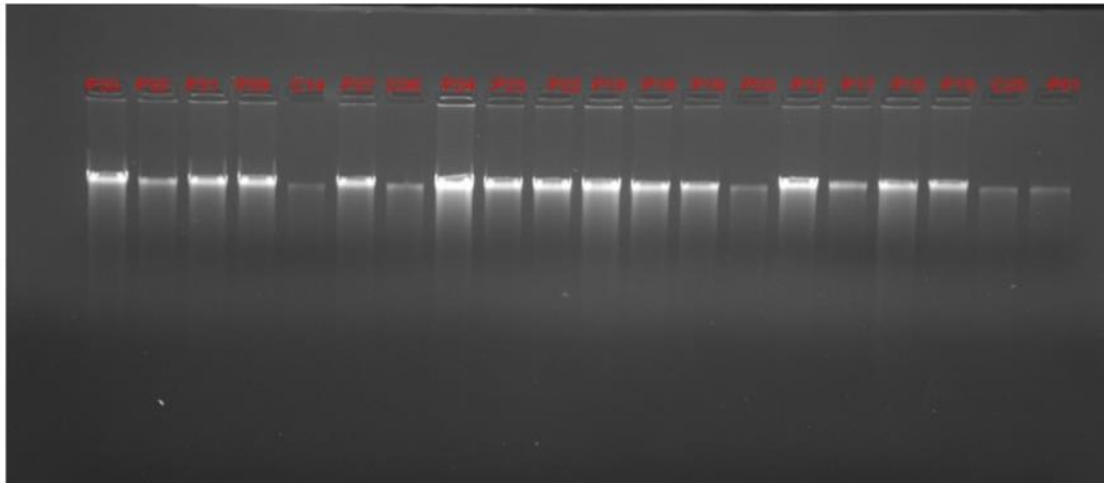
Fig. no 4.21. Agarose gel electrophoresis results using 0.8% agarose gel

Analysis of DNA purity and concentration isolated qualitatively can be seen based on the characteristic of the DNA bands in 0.8% agarose gel electrophoresis results
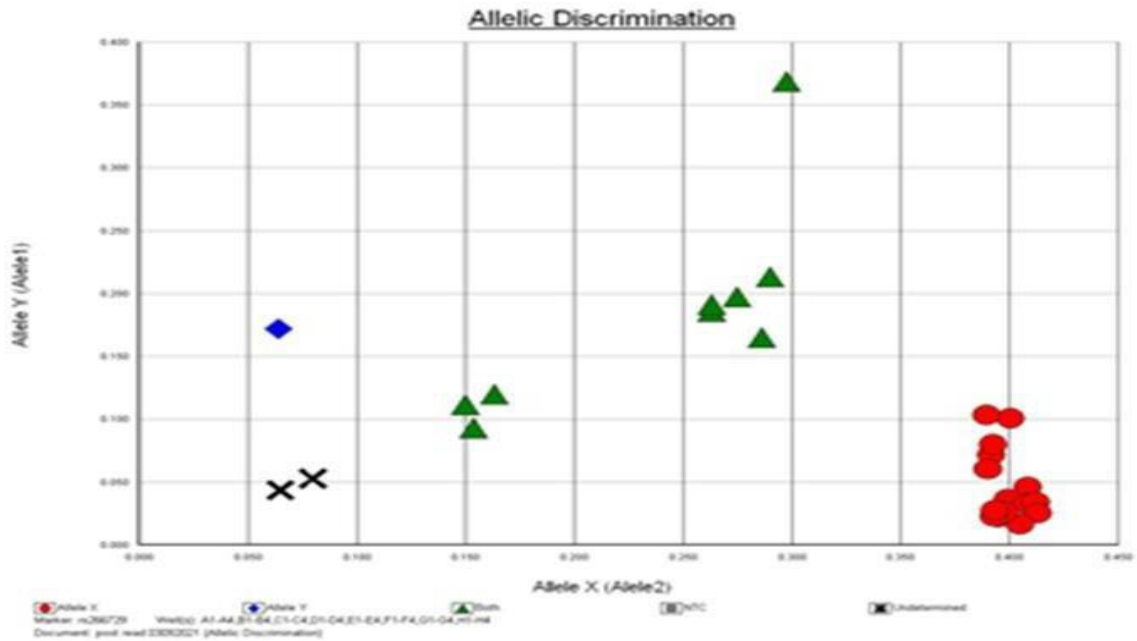
Fig no.4.22 Real time PCR based assays for SNP genotyping for ADIPOQ gene (rs266729)

Figure no. 4.22 Allelic discrimination plots obtained for rs266729 using KASP genotype assay on 96 samples. Allele X homozygote with FAM dye (FAM allele represented on X axis). Allele Y homozygote with HEX dye (HEX allele represented on Y axis). Both (X allele and Y allele) are heterozygote. Red and blue color represent homozygous genotype (CC&GG). The green color represents heterozygous genotype (CG).

DNA SEQUENCING RESULT OF ADIPOQ GENE

SINGLE NUCLEOTIDE POLYMORPHISM

Figure.no. 4.23. Verification of genotyping result by Sanger sequencing (rs266729 C/G)

DNA sequencing result obtained for rs266729 ADIPOQ gene using sanger sequencing – mutation observed C converted to G.
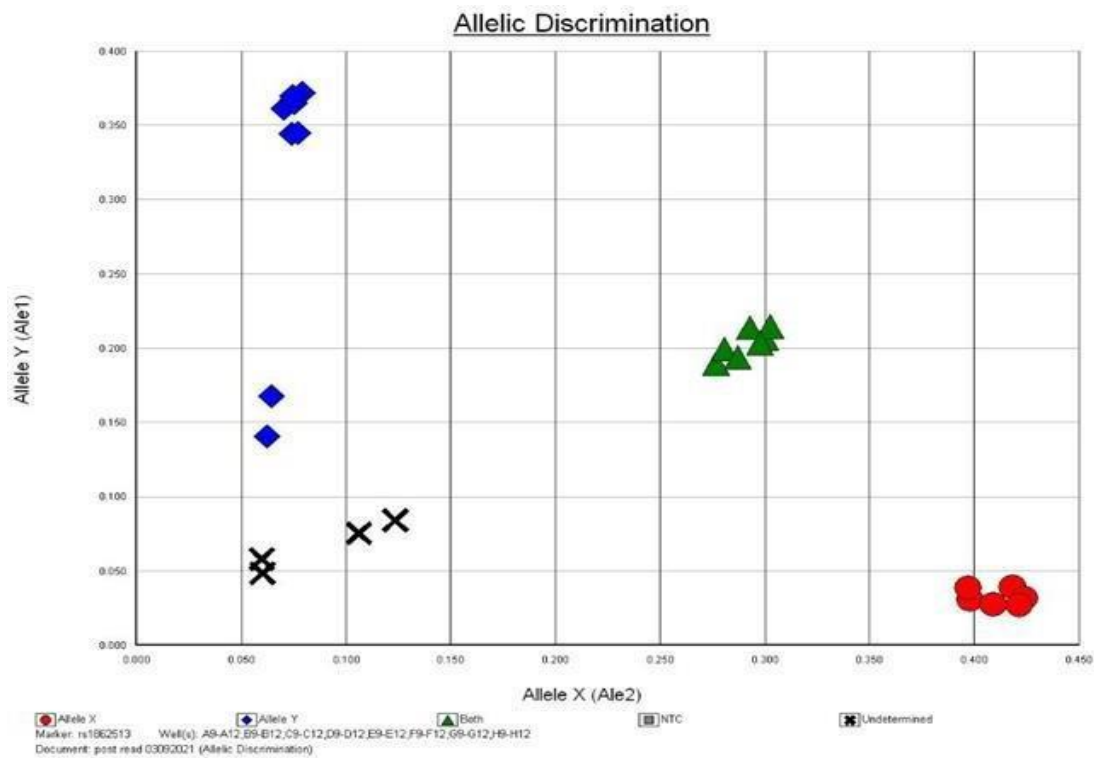
Figure.no. 4.24 Real time PCR based assays for SNP genotyping for RETN gene (rs1862513)

Allelic discrimination plots obtained for rs1862513 using KASP genotype assay on 96 samples. Allele X homozygote with FAM dye (FAM allele represented on X axis). Allele Y homozygote with HEX dye (HEX allele represented on Y axis). Both (X allele and Y allele) are heterozygote. Red and blue color represent homozygous genotype (CC&GG). The green color represents heterozygous genotype (CG).

DNA SEQUENCING RESULT OF RETN GENE

SNP ANALYSIS				
SAMPLES NAME	SNPID	MUTATION OCCURED/NOT	GENE POSITION	POSITION
0821_579_C1_RETN420_F_H10	rs1862513	YES	RETN420	299

SNP sequence : rs1862513

MUTATION OCCURED:C converted to G

Figure.no. 4.25. Verification of genotyping result by Sanger sequencing (rs1862513 C/G)

DNA sequencing result obtained for rs1862513 RETN gene using sanger sequencing. Mutation observed C converted to G.
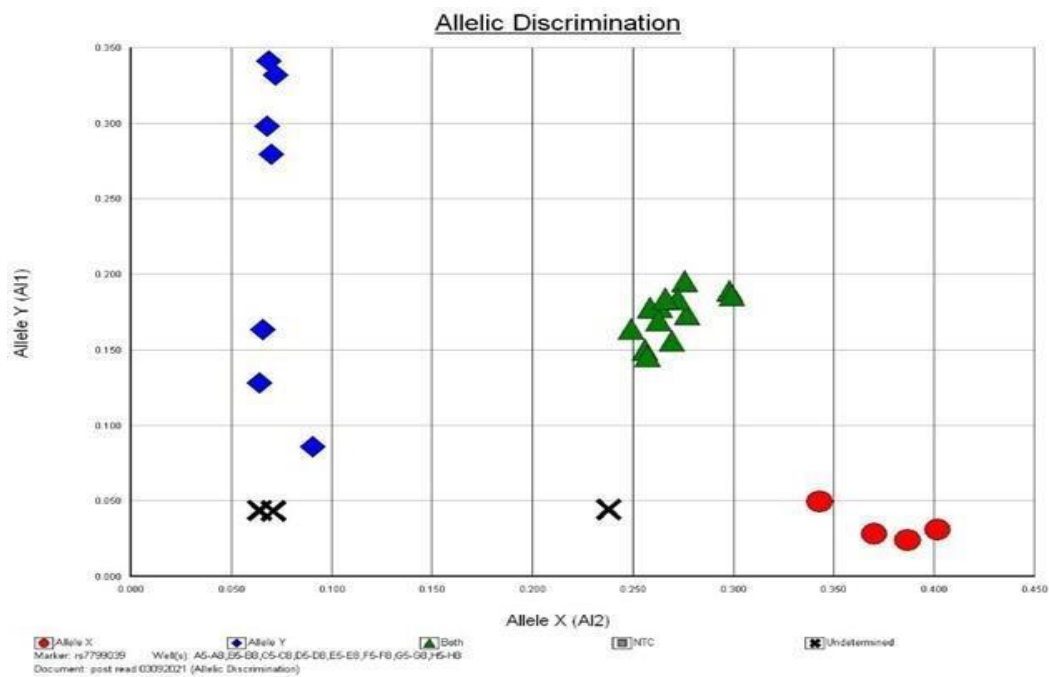
Fig.No. 4.26. Real time PCR based assays for SNP genotyping for LEP gene (rs7799039)

Allelic discrimination plots obtained for rs7799039 using KASP genotype assay on 96 samples. Allele X homozygote with FAM dye (FAM allele represented on X axis). Allele Y homozygote with HEX dye (HEX allele represented on Y axis). Both (X allele and Y allele) are heterozygote. Red and blue color represent homozygous genotype (GG&AA). The green color represents heterozygous genotype (GA).

SINGLE NUCLEOTIDE POLYMORPHISM

SNP ANALYSIS				
SAMPLES NAME	SNPID	MUTATION OCCURED/NOT	GENE POSITION	POSITION
0921_155_P61_LEP2548_F_F09	rs7799039	Yes	LEP2548(G>A)	63

G G G T T G C G C T G A T C C T C C C G C C T C A G T C T C C C

SNP sequence : rs7799039

G T C T C C C T A A G T G C T G A G A T G T T G C A G G A A G T C A G G G A C C C C G A A C A G A G A G

719.3997.63

Mutation Occured:G Converted to A
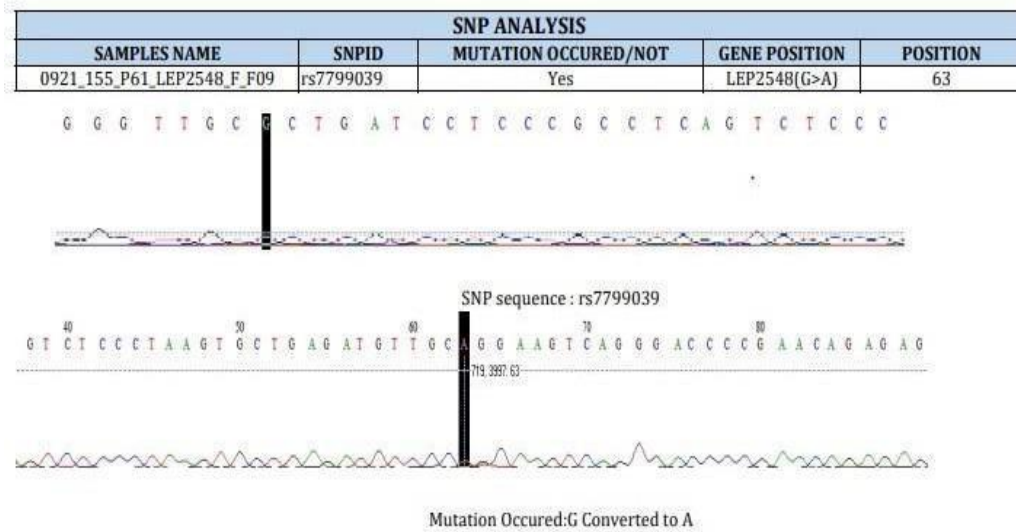
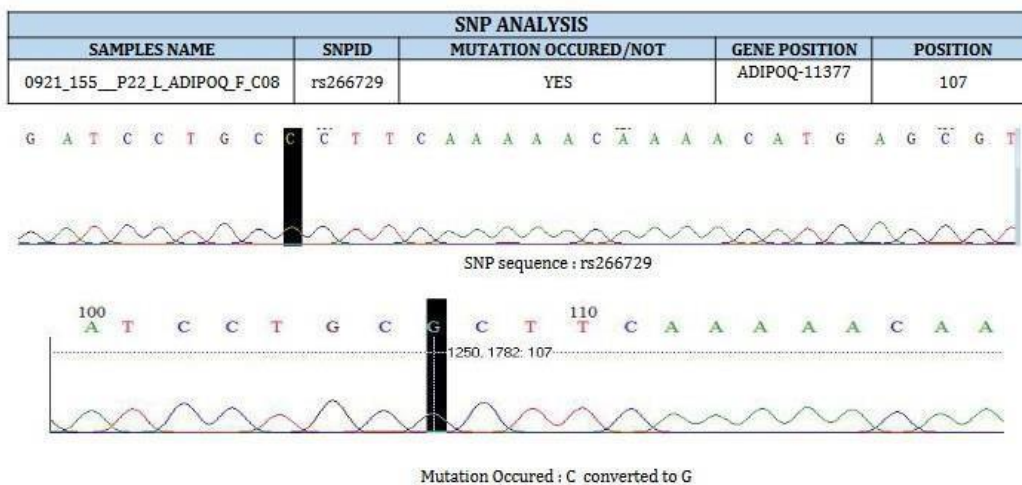**Figure.no. 4.27. Verification of genotyping result by Sanger sequencing (LEP)**

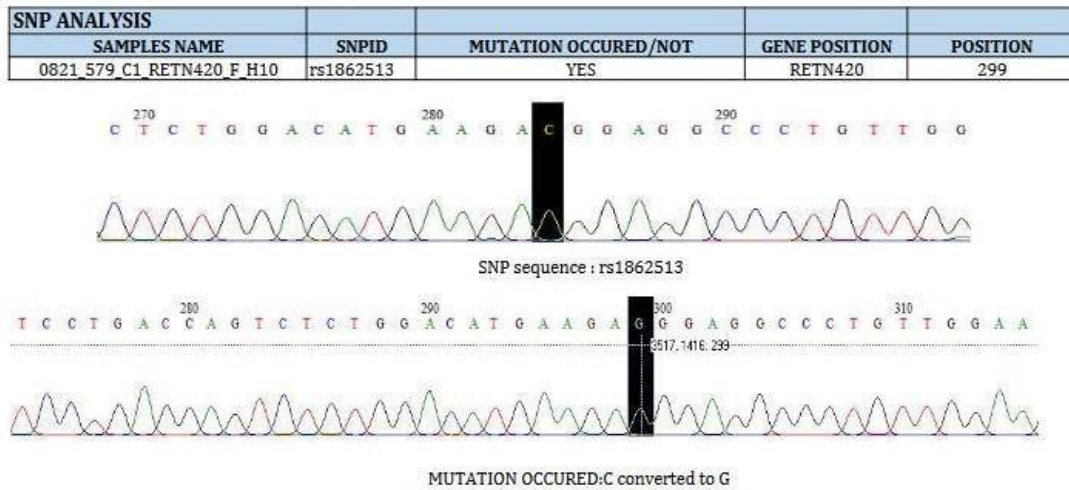DNA sequencing result obtained for rs7799039LEP gene using sanger sequencing. Mutation observed G converted to A.

Chapter-5

DISCUSSION

For diagnosing and managing prediabetes fasting insulin level is a promising method. (Alteman et al., 2018). One of the main causes for prediabetes is insulin resistance. (Cai et al., 2019). It was observed in the present study the state of insulin resistance may be an important factor in the development of normal glucose tolerance to prediabetic.

Fasting plasma glucose is not a reliable method as it was not able to detect almost all the cases of IGT in adolescents and young adults who were at a risk of MetS and diabetes. However, IGT was associated with increased IR and a dysfunctional beta cell of pancreas. They also pose a severe risk for cardiometabolic diseases. (Li et al., 2020).

In the current study, according to the level of HbA1c and OGTT, the study subjects were grouped as control group (N=150) and pre-diabetes group (N=150). Male subjects are dominant than female in non-diabetic group for T2DM (Rajmangal et al., 2020). The mean age for cases was 42.98±8.16 years and controls were 36.75±5.84 years which were in accordance (Diwan et al., 2018). Mean BMI for control group was 22.56±4. and T2DM (Study group) was 25.85±4.34 (Rajmangal et al., 2020). Mean Waist Circumference (WC) in T2DM group were more i.e. 99.51±3.65 cm than in control group i.e. 86.74±8.09 cm (Lalrindiki et al., 2019).

In the present study, serum resistin level elevated significantly among obese prediabetes than the non-obese prediabetes and control subjects (p<0.001). Although, significantly decreased A/L ratio were also observed in obese prediabetes subjects when compared to the rest.

Those cases with normal BMI showed higher levels of adiponectin than obese counterparts who were newly diagnosed with T2DM (Darabi et al., 2015). There is also an altered circulating adipokine patterns in obesity and these may be a risk factor to comorbid conditions like impaired glucose metabolism and T2DM (Van Gaal et al., 2006).

After adjusting for BMI and WC, the difference between these groups maintained, indicating that adiponectin is linked with BMI status in T2DM patients (Neuparth et al., 2013). There are known anti diabetic and anti-inflammatory effects of adiponectin resulting

obese patients with T2DM prone to more severe insulin resistance than those with normal BMI. (Fruhbeck et al., 2019).

Based on these facts it can be said that adiponectin can be a very good marker for risk evaluation of prediabetes and coronary artery disease (Stojanovic et al., 2015). Individuals above 60 years of age have adiponectin concentration highly dependent on obesity and T2DM (Coimbra et al., 2014). These outcomes suggest that adiponectin level depends on obesity rather than on T2DM which is a similar report to the current study.

Serum adiponectin and IR severity are inversely correlated (Banerjee et al., 2017). Similarly, there is also increasing evidence of is an inverse correlation between body weight and adiponectin levels in serum. (Rajković et al., 2014).

Patients who were recently diagnosed with T2DM had higher levels of leptin and lower adiponectin. (Zhou et al., 2016).

In the present study, decreased level of serum adiponectin was found in in obese prediabetes. In accordance with the result of Adami et al., (2016), they have demonstrated that there is lower adiponectin concentration in overweight people than obese diabetics. (Adami et al., 2016).

Hypoadiponectemia comes as a comorbid condition with T2DM but its levels are much lower than obese ones (Rajković et al., 2014). There is sequential increase in serum adiponectin in biliopancreatic diversion in non-remitter ones than those with remitter ones (Kocot et al., 2017).

It has been a popular interest for making thresholds for HbA1c, FPG and 2-hour OGTT based on different ethnicity in populations. (Echouffo-Tcheugui et al., 2011). There is a distort adipocytokine levels in patients with obesity and T2DM (Li et al., 2020).

American Diabetes Association (2019) defined that, "because the first elimination of alternative causes of hyperglycemia is often required for the diagnosis of T2DM It is not unexpected that the clinical manifestation, development, and therapeutic response to T2DM are so variable". T2DM constitutes approximately 90% of all diagnoses of diabetes (Li et al., 2020).

In a study done in 6884 Thai lands, the results showed the misdiagnoses of prediabetes by FPG in 46.3% cases whereas the cases with OGTT were only 18.9%. (Aekplakorn et al.,

2015). Increased WC and WHR in diabetic group can be linked to such individuals having store fat levels" (Lalrindiki et al., 2019).

In present study it was observed that mean serum adiponectin level and mean serum leptin levels were compared with the incidence of impaired blood glucose level in the case study population. Adhikari et al., (2018) reported significantly lower levels of adiponectin in metabolic syndrome.

In the current study, leptin is negatively correlated with HDL while other anthropometric indices markers of insulin resistance and triglycerides had positive correlation similar to the study reported by Amita yadav et al., (2011).

Those who are carrying the GG genotype of rs2241766 are shown to have higher levels of serum adiponectin than patients who carry the TT or the TG genotypes of the SNP (Calkin and Allen 2006). rs1501299 site is an intronic variant of the ADIPOQ gene" (Marcovecchio et al., Tripathi and Srivastava (2006) have conducted research in various communities globally to investigate the link between two common single nucleotide polymorphisms (SNPs), namely rs266729 and rs1501299, in the ADIPOQ gene and the susceptibility to T2DM. In the current study there was no significant association between Adiponectin level with Adiponectin SNP in control group ($\chi2$ = 1.549, p>0.05) was observed.

Several studies have linked rs1501299 to differences in serum adiponectin. Individuals with the T allele of this SNP was more likely to develop hyperglycemia and/or the metabolic syndrome (Nasri et al., 2015). Due to an association of the T allele with lower serum adiponectin levels (Zhou et al., 2016). In the present study, there was a significant association between Adiponectin level with Adiponectin Single Nucleotide Polymorphisms in non-obese pre-diabetic group ($\chi2$ = 16.684, p<0.001) was observed.

There is a depletion of adiponectin levels in all T2DM patients, and also a significant increase in levels in females vs. males" (Picu et al., 2013). We have also found a significant reduction in serum adiponectin concentration in the case of obese samples when compared with the normal healthy control group whose body weight were normal. In the present meta-analysis, we found a significant association between the TT genotype of + 276 G > T rs1501299 and increased risk of T2DM (Raghushaker et al., 2013).

Insulin resistance has been identified as a factor contributing to the increased likelihood of the rs2241766 variant in the ADIPOQ gene being associated with various

metabolic disorders (Dong et al., 2020). In the present study was demonstrated the distribution and association of Genotype and Allele of Adiponectin polymorphism between pre diabetic and control group. A correlation has been found and established between the risk for T2DM and rs2241766 variant in the ADIPOQ gene. The co dominant, dominant, recessive and alleles found to have statistically significant association for cases as compared to controls (Dong et al., 2020).

Adipose tissue secretes adipocytokines, which play a crucial part in our body's homeostatic equilibrium and also in the maintenance of energy balance. All the three adipokines i.e., "Leptin, resistin, and adiponectin" are all linked to diabetic pathophysiology and complications as it helps in the modulation of the insulin activity and also the inflammation (Bełtowski et al., 2006). A higher level of adiponectin lowered in diabetics is demonstrated by flabby and overweightness when compared to underweights, in case of both patients with diabetes mellitus and non-diabetic healthy humans" (Neuparth et al., 2013). No significant difference has been observed in the serum adiponectin concentration in the lean patients with diabetes mellitus and non-diabetic healthy humans however the serum concentration of adiponectin was found to be at higher level in case of fatty diabetics when compared to normal healthy obese candidates (Kocot et al., 2017).

The role of adipocytokines in pathophysiology has remained controversial since years (Lee et al., 2019). Leptin is said to be a marker for obesity and diabetes (Ghadge and Khaire et al., 2019). There is still a debate on blood concentration of leptin in diabetics and its association with other medical considerations (Tatti et al., 2001).

Among all the adipocytokines, adiponectin is not only the most important one but it also a key regulator of carbohydrate and fat metabolism (Cai et al., 2019). Another very significant adipocytokines which works as interpreter for the T2DM, insulin resistance as well as for obesity (Ghadge and Khaire et al., 2010). Serum concentration of leptin has been found a significant association with efficacy of insulin and prediabetes patients also and can be used as a screening biomarker tool to screen out prediabetes with normal healthy individuals (Wang et al., 2019).

In the current study, women's plasma leptin levels were discovered to be much higher than men's. These results are dependable with observations by another author (Susilowati et al., 2016). The association of serum leptin concentration and obesity is very well defined and very clear whereas the same between diabetes and blood concentration of leptin is under the shadow and not very well understood (Mohammadzadeh et al., 2013).

In case of Adiponectin-Leptin ratios, a ratio of 1.0 or higher is considered within the normal range. Ratios falling between 0.5 and 1.0 suggest a moderate to medium increase in cardio-metabolic risk, whereas ratios lower than 0.5 indicate a considerable elevation in cardiac and metabolic risk. (Fruhbeck et al., 2018). Observations of the current analysis point out that the total adiponectin and leptin ratio has been found significantly lowered in the case of overweight prediabetes groups when compared with healthy control group and normal weighted prediabetics group. In both obese and non-obese subjects, a negative weak correlation was observed between adiponectin-leptin ratio and fasting insulin resistance, whereas, positive poor association were observed in control group. Leptin concentrations in diabetics have not given consistent outcomes (Al-Hamod et al., 2014). The reason may be fault in selecting study groups as gender and obesity levels are said to be main causes determining leptin levels (Susilowati et al., 2016).

Kazmi et al., (2012) had observed slight fall down in leptin levels in both sex of diabetics having normal bodyweight giving results and was found aligned with the earlier findings. However, a study done by Rajković et al., (2014), have not demonstrated any discrepancy in serum leptin level between underweight T2DM patients and underweight control samples. Current study showed that there was no significant association between Adiponectin level with Adiponectin Single Nucleotide Polymorphisms in control group ($\chi2$ = 0.797, p>0.05). In both the univariate and multivariate analyses, they demonstrated that individuals with the heterozygous genetic constitutions of rs2167270 had a comparatively higher risk of developing prediabetics (Vasilescu et al., 2011).

If we compare leptin levels between overweight persons with diabetes and healthy controls the results have been inconclusive may be due to the different methodologies used to recruit study participants (Das et al., 2014). We were also found that those with newly diagnosed prediabetes and normal BMI showed lower serum leptin levels than obese patients. After accounting for BMI and waist circumference, this difference was still statistically significant.

Genotyping is done using real time PCR. Using Sanger sequencing method verification of genotyping was carried out. Moreover, it was observed that a similar pattern. Numerous SNPs have been identified in LEP and LEPR genes due to their highly polymorphic nature (Hoffstedt et al., 2002). There is association between LEP G2548A SNP and T2DM as well as related metabolic traits. An increase in obesity is comorbid with LEP G2548A and LEPR Q223R polymorphisms (Ying et al., 2009). Blood

concentration of leptin present in obese diabetes mellitus patients and that of normal control group show no differences and a similar observation is observed in T2DM patients (Vasilescu et al., 2011). An increased leptin levels in T2DM candidates compared to healthy individuals as a contradiction was demonstrated by Das et al., (2013).

Similarly, the investigation revealed that a haplotype encompassing all three examined SNPs, incorporating the A allele of rs2167270, was associated with an increased predisposition to prediabetes. Conversely, another haplotype containing the predominant G allele exhibited a protective effect against the development of the disease. These findings collectively suggest that the minor A allele of rs2167270 is a high-risk variant potentially implicated in the pathophysiology of prediabetes. In the 5'-untranslated region of LEP where SNP was located. In the current study, the association of co dominant, dominant, recessive genotype and allele between occurrences and controls were observed statistically significant. Hansen et al., (2010) showed no difference in resistin levels according to body weight index. Also, there was no distinctions observed on the criterion of body weight when compared diabetic peoples and non-diabetics healthy peoples (Al-Suhaimi et al., 2013). The researchers concluded that resistin did not play a role in the metabolic alterations associated with insulin-independent diabetes. In the current study it was observed that there was no significant association between Adiponectin level with Adiponectin Single Nucleotide Polymorphisms in the control group ($\chi2 = 5.004$, p>0.05).

To correlate RETN variation and MS, a study in Thais found that SNPs −420C>G and +299G>A were not associated with an elevated risk of MS. There is a link between RETN −420C>G polymorphism and MS in this study. (Gupta et al., 2011). SNP −420C>G was associated with the increasing incidence of metabolic disease in populations of Italy as well as Japan as elaborated by Norata et al., (2007) and Miyamoto et al., (2009).

There was no significant association between resistin level with resistin Single Nucleotide Polymorphisms in non-obese pre-diabetic group ($\chi2 = 0.813$, p>0.05) was found in the current study. The presence of the RETN −420CC genotype and elevated plasma resistin levels is associated with an increased risk of T2DM patients, as indicated by RETN gene variants (Rathwa et al., 2017).

A polymorphism in RETN -638G/A DNA segment is inconsistent in the residents, as only GG genetic constitution was exhibit in all the models. They also didn't find any

link between RETN -358 G/A with T2DM patients nor with any further considerations. Comparable conclusions are seen in Malaysian population" (Apalasamy et al., 2015). We have also not found any significant link between resistin level with resistin Single Nucleotide Polymorphisms in obese prediabetic group ($\chi 2 = 1.,000$ p>0.05).

They have observed discrepancies in blood level of resistin in obese diabetic patients and non-diabetic healthy groups (Mohammadzadeh et al., 2013). However, there is no significant distinction were seen in diabetes and non-diabetes studies related to resistin (Al Sheikh 2017). Significantly higher resistin concentration in obese diabetics vs. obese non-diabetics, as well as increased resistin level in obese diabetics and non-diabetics vs. slim healthy persons". (Mabrouk et al., 2013) in his study revealed contradiction as there was very meagre increase in resistin levels in obese diabetics when compared to controls. On the contrary, individuals carrying the SNP −420C>G variant at the RETN locus did not demonstrate any correlation with susceptibility to multiple sclerosis in Caucasian populations (Qasim et al., 2009). A very equivalent results was found in Malaysian men (Lau et al., 2011) and these results were confirmed by our study. If we talk about links between the RETN +299G>A polymorphism and MS, there is a study in Japanese cohort with the A allele of SNP +299G>A showing association with increased risk of MS (Miyamoto et al., 2009). In the current study, the genotype and allele frequencies of rs1862513 promoter region RETN gene polymorphism in prediabetes and healthy control were observed.

Increased resistin levels are correlated with RETN 420 C/G polymorphism (Osawa et al., 2009). "There are also investigations revealing association of RETN genetic variations and resistin levels with BMI, FBG, and plasma lipid profile. (Rathwa et al., 2017). It was found CC of RETN-420C/G SNP to be significantly associated with metabolic risk factors marked by higher FBG, BMI, and TC in T2DM patients. The CC genotype is associated with other factors as observed in various populations.

In the current study, the frequency of the CC, CG and GG. genotype of rs1862513 were 36.7%,43.3%, and 20% in prediabetes case and 40%, 39.3%, 20.7% in healthy control respectively. The allele frequency of C and G were 58.3% and 41.7% in prediabetes and 59.7% and 49.3% in healthy control respectively. There was no significant link between homozygous CC and heterozygous CG genotype with prediabetes was noted. Moreover,

CG allele frequency of rs1862513 were also did not showed any association with prediabetes as compared to healthy controls were also observed.

CONCLUSION

The findings of the current study suggested that, in patients with newly diagnosed prediabetes, adipocytokine concentrations (leptin, resistin, and adiponectin) differed between patients who had normal BMI and those who were obese. Serum leptin levels increased significantly among obese with prediabetes when compared to non-obese prediabetes and healthy subjects in the control group. Serum resistin levels among non-obese prediabetic and healthy participants in the control group research subjects showed a similar outcome.

Obesity and MS are characterized by increased and decreased levels of leptin and adiponectin, respectively. This translates in a reduction in the adiponectin/leptin ratio which is indicative of a dysfunctional adipose tissue. A significantly decreased adiponectin leptin ratio was observed among obese prediabetes subjects when compared to the rest ($p < 0.001$). Study subjects with prediabetes who were obese showed a disturbed adipocytokine profile in the form of a significantly increased leptin concentration and reduced adiponectin level, compared with patients with prediabetes who had normal BMI.

There was a significant association between adiponectin level, leptin level, and gene polymorphism of ADIPOQ 11377(C>G) ($\chi 2=16.84$, $p=0.001$), LEP2548 (G>A) ($\chi 2=7.725$, $p=0.02$) in the non-obese prediabetic group, and not significant association between resistin level and RETN 420 polymorphism ($\chi 2=12.46$, $p=0.66$). It was observed that, a significant link between adiponectin level, leptin level, and gene polymorphism of ADIPOQ

11377(C>G) ($\chi 2$=12.46, p=0.002), LEP2548 (G>A) ($\chi 2$=6.97, p=0.03) in the obese prediabetic group, and not a significant association between resistin level and RETN 420 polymorphism ($\chi 2$=1, p=0.61). The association of all genotypes and allele polymorphism of adiponectin and leptin was found highly statistically significant, whereas resistin did not show any significant association.

The present study revealed that, plasma adiponectin levels and ADIPOQ C>G polymorphism have an association among study subjects with prediabetes. Similarly, the plasma leptin levels and LEP2548 G>A polymorphism also showed an associated between study subjects with prediabetes when compared to the rest.

From these findings and results, it was concluded that the obtained results suggest that adipokine profile and insulin resistance may useful markers in the identification of individuals with risk of developing metabolic syndrome as well as predictors of prediabetes. Our findings indicate that plasma adiponectin level and ADIPOQrs266729C>G polymorphism and plasma leptin level and LEP2548- rs7799039 G>A polymorphism may contribute to the genetic risk of prediabetes and provide preliminary data useful in genetic screening for prediabetes among the Kerala population. Future studies are needed for the knowing the pathophysiology for regulating adipokines among those with risk of prediabetes and obesity, this could be a promising novel approach for managing metabolic disorders.

REFERENCES

Abbasi A, Peelen LM, Corpeleijn E, Van Der Schouw YT, Stolk RP, Spijkerman AM, Moons KG, Navis G, Bakker SJ, Beulens JW. Prediction models for risk of developing type 2 diabetes: systematic literature search and independent external validation study. Bmj. 2012 Sep 18;345.

Abdul-Ghani MA, Jenkinson CP, Richardson DK, Tripathy D, DeFronzo RA. Insulin secretion and action in subjects with impaired fasting glucose and impaired glucose tolerance: results from the Veterans Administration Genetic Epidemiology Study. Diabetes. 2006 May 1;55(5):1430-5.

Abdul-Ghani MA, Molina-Carrion M, Jani R, Jenkinson C, DeFronzo RA. Adipocytes in subjects with impaired fasting glucose and impaired glucose tolerance are resistant to the anti-lipolytic effect of insulin. Acta diabetologica. 2008 Sep;45(3):147-50.

Abdul-Ghani MA, Stern MP, Lyssenko V, Tuomi T, Groop L, DeFronzo RA. Minimal contribution of fasting hyperglycemia to the incidence of type 2 diabetes in subjects with normal 2-h plasma glucose. Diabetes care. 2010 Mar 1;33(3):557-61.

Abdul-Ghani, M. A., & DeFronzo, R. A. (2009). Pathophysiology of prediabetes. Current diabetes reports, 9(3), 193-199.

Adami GF, Gradaschi R, Andraghetti G *et al*.,: Serum leptin and adiponectin concentration in type 2 diabetes patients in the short and long term following biliopancreatic diversion. Obes Surg, 2016; 26(10): 2442–48.

Adhikari P, Nagtilak S, Parashar P. Association of Adiponectin with Components of Metabolic Syndrome in Western U. Int J Med Sci Innov Res. 2018;3(6):215–21.

Adiga, Ushaa,*; Banawalikar, Nanditb; Mayur, Sriprajnab; Bansal, Radhikab; Ameera, Nafeesathb; Rao, Sudhindrac Association of insulin resistance and leptin receptor gene polymorphism in type 2 diabetes mellitus., April 2021 - Volume 84 - Issue 4 - p 383-388

Aekplakorn W, Tantayotai V, Numsangkul S, Sripho W, Tatsato N, Burapasiriwat T, Pipatsart R, Sansom P, Luckanajantachote P, Chawarokorn P, Thanonghan A. Detecting prediabetes and diabetes: agreement between fasting plasma glucose and oral glucose tolerance test in Thai adults. Journal of diabetes research. 2015 Oct;2015.

Aeschbacher S, Schoen T, Clair C, Schillinger P, Schönenberger S, Risch M, Risch L, Conen D. Association of smoking and nicotine dependence with pre-diabetes in young and healthy adults. Swiss medical weekly. 2014;144.

Al Sheikh MH. The determinants of leptin levels in diabetic and nondiabetic Saudi Males. International journal of endocrinology. 2017 Mar 1;2017.

Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith Jr SC. Harmonizing the metabolic syndrome: a joint interim statement of the international diabetes federation task force on epidemiology and prevention; national heart, lung, and blood institute; American heart association; world heart federation; international atherosclerosis society; and international association for the study of obesity. Circulation. 2009 Oct 20;120(16):1640-5.

Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. Diabetic medicine. 1998 Jul;15(7):539-53.

Al-Daghri NM, Al-Attas OS, Alokail MS, Alkharfy KM, Hussain T. Adiponectin gene variants and the risk of coronary artery disease in patients with type 2 diabetes. Molecular biology reports. 2011 Aug;38(6):3703-8.

Alexander CM, Landsman PB, Grundy SM. Metabolic syndrome and hyperglycemia: congruence and divergence. American Journal of Cardiology. 2006 Oct 1;98(7):982-5.

Alfaqih MA, Khader YS, Al-Dwairi AN, Alzoubi A, Al-Shboul O, Hatim A. Lower levels of serum adiponectin and the T allele of rs1501299 of the ADIPOQ gene are protective against polycystic ovarian syndrome in Jordan. Korean journal of family medicine. 2018 Mar;39(2):108.

Al-Hamodi Z, Al-Habori M, Al-Meeri A, Saif-Ali R. Association of adipokines, leptin/adiponectin ratio and C-reactive protein with obesity and type 2 diabetes mellitus. Diabetology & metabolic syndrome. 2014 Dec;6(1):1-8.

Alharbi KK, Abudawood M, Khan IA. Amino-acid amendment of arginine-325-tryptophan in rs13266634 genetic polymorphism studies of the SLC30A8 gene with type 2 diabetes-mellitus patients featuring a positive family history in the Saudi population. Journal of King Saud University-Science. 2021 Jan 1;33(1):101258.

Al-Harithy RN, Al-Zahrani MH. The adiponectin gene, ADIPOQ, and genetic susceptibility to colon cancer. Oncology letters. 2012 Jan 1;3(1):176-80.

Alimi M, Goodarzi MT, Nekoei M. Association of ADIPOQ rs266729 and rs1501299 gene polymorphisms and circulating adiponectin level with the risk of type 2 diabetes in a population of Iran: a case-control study. Journal of Diabetes & Metabolic Disorders. 2021 Jun;20(1):87-93.

Aljanabi MA, Alfaqih MA, Khanfar M, Amarin ZO, Elsalem L, Saadeh R, Al-Mughales F. Leptin and the GA genotype of rs2167270 of the LEP gene increase the risk of prediabetes. Biomedical Reports. 2021 May 1;14(5):1-8.

Al-Saeed AH, Constantino MI, Molyneaux L, D'Souza M, Limacher-Gisler F, Luo C, Wu T, Twigg SM, Yue DK, Wong J. An inverse relationship between age of type 2 diabetes onset and complication risk and mortality: the impact of youth-onset type 2 diabetes. Diabetes care. 2016 May 1;39(5):823-9.

Al-suhaimi A, Al-kulaifi M, Shehzad A. Serum adipokines metabolic function and immunological correlations in treated type 2 diabetes mellitus and obese subjects. World Applied Sciences Journal, 2013; 22(7): 933–38

Altemani, A. Alamri, M. Ahmed, M. Al Garbo, T. Alharbi, R. Al-Rasheed, S. Alshagraawi, I. Alzilai, F. Alotaibi, A. Al-shahrani, Prediabetes and serum insulin levels. Int. J. Community. Med. Public Health. 5(5) (2018) 1684-1689.

Altman J. Weight in the balance. Neuroendocrinology. 2002;76(3):131-6.

American Diabetes Association: 2 Classification and diagnosis of diabetes: standards of medical care in diabetes-2019. Diabetes Care 2019;42(Suppl 1):S13–28.

Amitani M, Asakawa A, Amitani H, Inui A. The role of leptin in the control of insulin-glucose axis. Frontiers in neuroscience. 2013 Apr 8;7:51.

Ang, G.Y., 2020. Age of onset of diabetes and all-cause mortality. World Journal of Diabetes, 11(4), p.95.

Apalasamy, S. Rampal, A. Salim, F.M. Moy, T.T. Su, H.A. Majid, A. Bulgiba, Z. Mohamed, Polymorphisms of the resistin gene and their association with obesity and resistin levels in Malaysian malays, Biochem. Genet. 53 (4–6) (2015 Jun 1) 120–131.

Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa JI, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochemical and biophysical research communications. 1999 Apr 2;257(1):79-83.

Arner P. Insulin resistance in type 2 diabetes-role of the adipokines. Current molecular medicine. 2005 May 1;5(3):333-9.

Aroda VR, Ratner R. Approach to the patient with prediabetes. J Clin Endocrinol Metab 2008;93(9):3259–65

Ashby JP, Frier BM. Circulating C-peptide: measurement and clinical application. Annals of clinical Biochemistry. 1981 May;18(3):125-30.

Avery PJ, Patel SK, Ibrahim IM, Walker M, Keavney BD. Common variation in the adiponectin gene has an effect on systolic blood pressure. Journal of human hypertension. 2011 Dec;25(12):719-24.

Azuma K, Oguchi S, Matsubara Y, Mamizuka T, Murata M, Kikuchi H, Watanabe K, Katsukawa F, Yamazaki H, Shimada A, Saruta T. Novel resistin promoter polymorphisms: association with serum resistin level in Japanese obese individuals. Hormone and metabolic research. 2004 Aug;36(08):564-70.

Badersten A, Nilvéus R, Egelberg J. Effect of nonsurgical periodontal therapy. I. Moderately advanced periodontitis. J Clin Periodontol. 1981;8:57–72.

Balion CM, Raina PS, Gerstein HC, *et al*.,. Reproducibility of impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) classifi cation: a systematic review. Clin Chem Lab Med 2007; 45: 1180–85.

Balkau B., Mhamdi L., Oppert J.M., Nolan J., Golay A., Porcellati F., Laakso M., Ferrannini E., EGIR-RISC Study Group Physical activity and insulin sensitivity: The RISC study. Diabetes. 2008;57:2613–2618. doi: 10.2337/db07-1605.

Banerjee A, Khemka VK, Roy D, Poddar J, Roy TK, Karnam SA. Role of serum adiponectin and vitamin D in prediabetes and diabetes mellitus. Canadian journal of diabetes. 2017 Jun 1;41(3):259-65.

Bansal, N. (2015). Prediabetes diagnosis and treatment: A review. World journal of diabetes, 6(2), 296.

Barr EL, Boyko EJ, Zimmet PZ, Wolfe R, Tonkin AM, Shaw JE. Continuous relationships between non-diabetic hyperglycaemia and both cardiovascular disease and all-cause mortality: the Australian Diabetes, Obesity, and Lifestyle (AusDiab) study. Diabetologia. 2009 Mar;52(3):415-24.

Barrett-Connor E, Wingard DL. " Normal" blood glucose and coronary risk: Dose response effect seems consistent throughout the glycaemic continuum. BMJ. 2001 Jan 6;322(7277):5-6.

Barry E, Roberts S, Oke J, Vijayaraghavan S, Normansell R, Greenhalgh T. Efficacy and effectiveness of screen and treat policies in prevention of type 2 diabetes: systematic review and meta-analysis of screening tests and interventions. bmj. 2017 Jan 4;356.

Belkina AC, Denis GV. Obesity genes and insulin resistance. Current opinion in endocrinology, diabetes, and obesity. 2010 Oct;17(5):472.

Bełtowski J. Apelin and visfatin: unique "beneficial" adipokines upregulated in obesity? Med Sci Monit 2006;12:112–9.

Bender N, Allemann N, Marek D, Vollenweider P, Waeber G, Mooser V, Egger M, Bochud M. Association between variants of the leptin receptor gene (LEPR) and overweight: a systematic review and an analysis of the CoLaus study. PloS one. 2011 Oct 18;6(10):e26157.

Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nature medicine. 2001 Aug;7(8):947-53.

Bergman BC, Perreault L, Hunerdosse DM, Koehler MC, Samek AM, Eckel RH. Intramuscular lipid metabolism in the insulin resistance of smoking. Diabetes. 2009 Oct 1;58(10):2220-7.

Bertram MY, Vos T. Quantifying the duration of pre-diabetes. Australian and New Zealand journal of public health. 2010 Jun;34(3):311-4.

Bianchi C, Miccoli R, Bonadonna RC, Giorgino F, Frontoni S, Faloia E, Marchesini G, Dolci MA, Alviggi L, Gnasso A, Consoli A. Metabolic syndrome in subjects at high risk for type 2 diabetes: the genetic, physiopathology and evolution of type 2 diabetes (GENFIEV) study. Nutrition, Metabolism and Cardiovascular Diseases. 2011 Sep 1;21(9):699-705.

Bird A. Perceptions of epigenetics. Nature. 2007 May 24;447(7143):396.

Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Meigs JB, Bonadonna RC, Muggeo M. Population-based incidence rates and risk factors for type 2 diabetes in white individuals: the Bruneck study. Diabetes. 2004 Jul 1;53(7):1782-9.

Boumaiza I, Omezzine A, Rejeb J, Rebhi L, Ben Rejeb N, Nabli N, Ben Abdelaziz A, Bouslama A. Association between four resistin polymorphisms, obesity, and metabolic syndrome parameters in Tunisian volunteers. Genetic Testing and Molecular Biomarkers. 2012 Dec 1;16(12):1356-62.

Bruin JE, Petre MA, Lehman MA, Raha S, Gerstein HC, Morrison KM, Holloway AC. Maternal nicotine exposure increases oxidative stress in the offspring. Free Radical Biology and Medicine. 2008 Jun 1;44(11):1919-25.

Brunner EJ, Shipley MJ, Witte DR, Fuller JH, Marmot MG. Relation between blood glucose and coronary mortality over 33 years in the Whitehall Study. Diabetes care. 2006 Jan 1;29(1):26-31.

Bungau S, Behl T, Tit DM, Banica F, Bratu OG, Diaconu CC, Nistor-Cseppento CD, Bustea C, Aron RA, Vesa CM. Interactions between leptin and insulin resistance in patients with prediabetes, with and without NAFLD. Experimental and Therapeutic Medicine. 2020 Dec 1;20(6):1-.

Burnett MS, Devaney JM, Adenika RJ, Lindsay R, Howard BV. Cross-sectional associations of resistin, coronary heart disease, and insulin resistance. The Journal of Clinical Endocrinology & Metabolism. 2006 Jan 1;91(1):64-8.

Burtis CA, Ashwood ER. Tietz textbook of clinical chemistry. Amer Assn for Clinical Chemistry; 1994.

Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. Diabetes. 2003 Jan 1;52(1):102-10.

Buysschaert, M., & Bergman, M. (2011). Definition of prediabetes. Medical Clinics, 95(2), 289-297.

Cai X, Xia L, Pan Y, He D, Zhu H, Wei T, He Y. Differential role of insulin resistance and β-cell function in the development of prediabetes and diabetes in middle-aged and elderly Chinese population. Diabetology & metabolic syndrome. 2019 Dec;11(1):1-8.

Calabro P, Samudio I, Willerson JT, Yeh ET. Resistin promotes smooth muscle cell proliferation through activation of extracellular signal–regulated kinase 1/2 and phosphatidylinositol 3-kinase pathways. Circulation. 2004 Nov 23;110(21):3335-40.

Calkin AC, Allen TJ. Diabetes mellitus-associated atherosclerosis. American Journal of Cardiovascular Drugs. 2006 Jan;6(1):15-40.

Campagna D, Alamo A, Di Pino A, Russo C, Calogero AE, Purrello F, Polosa R. Smoking and diabetes: dangerous liaisons and confusing relationships. Diabetology & metabolic syndrome. 2019 Dec;11(1):1-2.

Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science. 1995 Jul 28;269(5223):546-9.

Carlsson S, Midthjell K, Grill V. Smoking is associated with an increased risk of type 2 diabetes but a decreased risk of autoimmune diabetes in adults: an 11-year follow-up of incidence of diabetes in the Nord-Trøndelag study. Diabetologia. 2004 Nov;47(11):1953-6.

Centers for Disease Control and Prevention. National diabetes statistics report: estimates of diabetes and its burden in the United States, 2014. Atlanta, GA: US Department of Health and Human Services. 2014 Jul;2014.

Cerami A, Koenig RJ. Hemoglobin AIc as a model for the development of the sequelae of diabetes mellitus. Trends in Biochemical Sciences. 1978 Apr 1;3(2):73-5.

Chan JM, Rimm EB, Colditz GA, Stampfer MJ, Willett WC. Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. Diabetes care. 1994 Sep 1;17(9):961-9.

Chang AM. Halter JB. Aging and insulin secretion. Am J Physiol Endocrinol Metab. 2003;284:E7-12.

Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives. Nature reviews endocrinology. 2012 Apr;8(4):228-36.

Chiolero A, Faeh D, Paccaud F, Cornuz J. Consequences of smoking for body weight, body fat distribution, and insulin resistance. The American journal of clinical nutrition. 2008 Apr 1;87(4):801-9.

Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes research and clinical practice. 2018 Apr 1;138:271-81.

Chouhan, A., Karma, A., Artani, N., & Parihar, D. (2016). Overview on cancer: role of medicinal plants in its treatment. World Journal of Pharmacy and Pharmaceutical Sciences, 5(5), 185-207.

Chu S, Ding W, Li K, Pang Y, Tang C. Plasma resistin associated with myocardium injury in patients with acute coronary syndrome. Circulation Journal. 2008;72(8):1249-53.

Clair C, Cohen MJ, Eichler F, Selby KJ, Rigotti NA. The effect of cigarette smoking on diabetic peripheral neuropathy: a systematic review and meta-analysis. Journal of general internal medicine. 2015 Aug;30(8):1193-203.

Coelho M, Oliveira T, Fernandes R. State of the art paper Biochemistry of adipose tissue: an endocrine organ. Archives of medical science. 2013 Mar 1;9(2):191-200.

Coimbra S, Brandão Proença J, Santos-Silva A, Neuparth MJ: Adiponectin, leptin, and chemerin in elderly patients with type 2 diabetes mellitus: A close linkage with obesity and length of the disease. Biomed Res Int, 2014; 2014: 701915

Colberg SR, Sigal RJ, Yardley JE, Riddell MC, Dunstan DW, Dempsey PC, Horton ES, Castorino K, Tate DF. Physical activity/exercise and diabetes: a position statement of the American Diabetes Association. Diabetes care. 2016 Nov 1;39(11):2065-79.

Colditz GA, Willett WC, Rotnitzky A, Manson JE. Weight gain as a risk factor for clinical diabetes mellitus in women. Annals of internal medicine. 1995 Apr 1;122(7):481-6.

Coutinho M, Gerstein HC, Wang Y, Yusuf S. The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies

of 95,783 individuals followed for 12.4 years. Diabetes care. 1999 Feb 1;22(2):233-40.

Cowie CC, Rust KF, Byrd-Holt DD, Gregg EW, Ford ES, Geiss LS, Bainbridge KE, Fradkin JE. Prevalence of diabetes and high risk for diabetes using A1C criteria in the US population in 1988–2006. Diabetes care. 2010 Mar 1;33(3):562-8.

Cui M, Gao Y, Zhao Y, Pang H, Chen L, Wang Z, Zhao L, Li M. Association between adiponectin gene polymorphism and environmental risk factors of type 2 diabetes mellitus among the Chinese population in Hohhot. BioMed Research International. 2020 Jun 22;2020.

Cullmann M, Hilding A, Östenson CG. Alcohol consumption and risk of pre-diabetes and type 2 diabetes development in a Swedish population. Diabetic Medicine. 2012 Apr;29(4):441-52.

Cusi K, Orsak B, Bril F, Lomonaco R, Hecht J, Ortiz-Lopez C, Tio F, Hardies J, Darland C, Musi N, Webb A. Long-term pioglitazone treatment for patients with nonalcoholic steatohepatitis and prediabetes or type 2 diabetes mellitus: a randomized trial. Annals of internal medicine. 2016 Sep 6;165(5):305-15.

D'Aiuto F, Parkar M, Andreou G, Suvan J, Brett PM, Ready D, et al.,. Periodontitis and systemic inflammation: Control of the local infection is associated with a reduction in serum inflammatory markers. J Dent Res. 2004;83:156–60.

Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, Lin JK, Farzadfar F, Khang YH, Stevens GA, Rao M. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2· 7 million participants. The lancet. 2011 Jul 2;378(9785):31-40.

Darabi H, Raeisi A, Kalantarhormozi MR, et al.,. Adiponectin as a protective factor against the progression toward type 2 diabetes mellitus in postmenopausal women. Medicine (Baltimore) 2015;94:e1347.

Das P, Bhattacharjee D, Bandyopadhyay SK, Bhattacharya G, Singh R. Association of obesity and leptin with insulin resistance in type 2 diabetes mellitus in Indian population. Indian J Physiol Pharmacol. 2013 Jan 1;57(1):45-50.

Davies TT, Graue M, Igland J, Tell GS, Birkeland KI, Peyrot M, Haltbakk J. Diabetes prevalence among older people receiving care at home: associations with symptoms, health status and psychological well-being. Diabetic Medicine. 2019 Jan;36(1):96-104.

De Luis DA, Izaola O, Primo D, Aller R. Relation of a variant in adiponectin gene (rs266729) with metabolic syndrome and diabetes mellitus type 2 in adult obese subjects. European Review for Medical & Pharmacological Sciences. 2020 Oct 15;24(20).

De Pablos-Velasco PL, Martinez-Martin FJ, Rodríguez-Pérez F. Prevalence of obesity in a Canarian community. Association with type 2 diabetes mellitus: the Guia Study. European Journal of Clinical Nutrition. 2002 Jun;56(6):557-60.

DeFronzo RA. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes. 2009 Apr 1;58(4):773-95.

Degawa-Yamauchi M, Bovenkerk JE, Juliar BE, Watson W, Kerr K, Jones R, Zhu Q, Considine RV. Serum resistin (FIZZ3) protein is increased in obese humans. The Journal of Clinical Endocrinology & Metabolism. 2003 Nov 1;88(11):5452-5.

DeJesus RS, Breitkopf CR, Rutten LJ, Jacobson DJ, Wilson PM, Sauver JS. Incidence rate of prediabetes progression to diabetes: modeling an optimum target group for intervention. Population health management. 2017 Jun 1;20(3):216-23.

Deng Y, Scherer PE. Adipokines as novel biomarkers and regulators of the metabolic syndrome. Annals of the New York Academy of Sciences. 2010 Nov;1212(1):E1-9.

Denroche HC, Huynh FK, Kieffer TJ. The role of leptin in glucose homeostasis. Journal of diabetes investigation. 2012 Apr;3(2):115-29.

Dhingra R, Sullivan L, Jacques PF, Wang TJ, Fox CS, Meigs JB, D'Agostino RB, Gaziano JM, Vasan RS. Soft drink consumption and risk of developing cardiometabolic risk factors and the metabolic syndrome in middle-aged adults in the community. Circulation. 2007 Jul 31;116(5):480-8.

Diamond J. Diabetes in india. Nature. 2011 Jan;469(7331):478-9.

Diwan AG, Kuvalekar AA, Dharamsi S, Vora AM, Nikam VA, Ghadge AA. Correlation of serum adiponectin and leptin levels in obesity and type 2 diabetes mellitus. Indian journal of endocrinology and metabolism. 2018 Jan;22(1):93.

Doll R, Peto R, Boreham J, Sutherland I. Mortality in relation to smoking: 50 years' observations on male British doctors. Bmj. 2004 Jun 24;328(7455):1519.

Dong Y, Huang G, Wang X, Chu Z, Miao J, Zhou H. Meta-analysis of the association between adiponectin SNP 45, SNP 276, and type 2 diabetes mellitus. PLoS One. 2020 Oct 22;15(10):e0241078.

Doria A, Patti ME, Kahn CR. The emerging genetic architecture of type 2 diabetes. Cell metabolism. 2008 Sep 3;8(3):186-200.

Dormandy JA, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK, Skene AM, Tan MH, Lefèbvre PJ, Murray GD, Standl E. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. The Lancet. 2005 Oct 8;366(9493):1279-89.

Duarte SF, Francischetti EA, Genelhu VA, Cabello PH, Pimentel MM. LEPR p.Q223R, beta3-AR p.W64R and LEP c.-2548G>A gene variants in obese Brazilian subjects. Genet Mol Res. 2007;6(4):1035-1043.

Dullaart RP, Kappelle PJ, Dallinga-Thie GM. Carotid intima media thickness is associated with plasma adiponectin but not with the leptin: adiponectin ratio

Dunmore, S.J. and Brown, J.E., 2013. The role of adipokines in b-cell failure of type 2 diabetes. J Endocrinol, 216(1), pp.37-45.

Dziemidok P., Kiełczykowska M., Hordyjewska A., Szcześniak G., Musik I. Adipokine Profile in Patients with Type 2 Diabetes Depends on Degree of Obesity. Med Sci. Monit. 2017;23:4995–5004. doi: 10.12659/MSM.904318.

Echouffo-Tcheugui JB, Selvin E. Prediabetes and what it means: the epidemiological evidence. Annual Review of Public Health. 2021 Apr 1;42:59-77.

Eissa AT. Investigate the relation between Adiponectin gene variants and cardiovascular comorbidities and diabetes. International Journal of Health Sciences. 2016 Apr;10(2):183.

Ekblad LL, Rinne JO, Puukka P, Laine H, Ahtiluoto S, Sulkava R, Viitanen M, Jula A. Insulin resistance predicts cognitive decline: an 11-year follow-up of a nationally representative adult population sample. Diabetes care. 2017 Jun 1;40(6):751-8.

Ekelund U, Brage S, Griffin SJ, Wareham NJ. Objectively measured moderate-and vigorous-intensity physical activity but not sedentary time predicts insulin resistance in high-risk individuals. Diabetes care. 2009 Jun 1;32(6):1081-6.

Emilsson V, Liu YL, Cawthorne MA, Morton NM, Davenport M. Expression of the functional leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion. Diabetes. 1997 Feb 1;46(2):313-6.

Engert JC, Vohl MC, Williams SM, Lepage P, Loredo-Osti JC, Faith J, Doré C, Renaud Y, Burtt NP, Villeneuve A, Hirschhorn JN. 5′ flanking variants of resistin are associated with obesity. Diabetes. 2002 May 1;51(5):1629-34.

Epifano L, Di Vincenzo A, Fanelli C, Porcellati E, Perriello G, De Feo P, Motolese M, Brunetti P, Bolli GB. Effect of cigarette smoking and of a transdermal nicotine delivery system on glucoregulation in type 2 diabetes mellitus. European journal of clinical pharmacology. 1992 Sep;43(3):257-63.

Ervin RB. Prevalence of metabolic syndrome among adults 20 years of age and over, by sex, age, race and ethnicity, and body mass index: United States, 2003-2006 National Health Statistics Reports, vol. 13, pp. 1–7, 2009.

Esposito K, Maiorino MI, Bellastella G, Panagiotakos DB, Giugliano D. Mediterranean diet for type 2 diabetes: cardiometabolic benefits. Endocrine. 2017 Apr;56(1):27-32.

Euser SM. A prospective analysis of elevated fasting glucose levels and cognitive function in older people: results from PROSPER and the Rotterdam Study. Diabetes. 2010;59:1601-7.

Ezenwaka CE, Kalloo R. Caribbean female patients with type 2 diabetes mellitus have lower serum levels of adiponectin than nondiabetic subjects. Neth J Med. 2005 Feb 1;63(2):64-9.

Faerch K, Vistisen D, Johansen NB, Jørgensen ME. Cardiovascular risk stratification and management in pre-diabetes. Current diabetes reports. 2014 Jun;14(6):1-8.

Fasshauer M, Paschke R. Regulation of adipocytokines and insulin resistance. Diabetologia. 2003 Dec;46(12):1594-603.

Ferrannini E. Definition of intervention points in prediabetes. The lancet Diabetes & endocrinology. 2014 Aug 1;2(8):667-75.

Ford ES, Zhao G, Li C. Pre-diabetes and the risk for cardiovascular disease: a systematic review of the evidence. Journal of the American College of Cardiology. 2010 Mar 30;55(13):1310-7.

Forouhi NG, Balkau B, Borch-Johnsen K, Dekker J, Glumer C, Qiao Q, Spijkerman A, Stolk R, Tabac A, Wareham NJ. The threshold for diagnosing impaired fasting glucose: a position statement by the European Diabetes Epidemiology Group. Diabetologia. 2006 May;49(5):822-7.

Frati AC, Iniestra F, Ariza CR. Acute effect of cigarette smoking on glucose tolerance and other cardiovascular risk factors. Diabetes care. 1996 Feb 1;19(2):112-8.

Fried SK, Bunkin DA, Greenberg AS. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. The Journal of Clinical Endocrinology & Metabolism. 1998 Mar 1;83(3):847-50.

Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature. 1998 Oct;395(6704):763-70.

Friedman JM. Modern science versus the stigma of obesity. Nature medicine. 2004 Jun;10(6):563-9.

Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE, Lodish HF. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proceedings of the National Academy of Sciences. 2001 Feb 13;98(4):2005-10.

Fruhbeck G, Catalán V, Rodríguez A, *et al*.,. Adiponectin–leptin ratio: a promising index to estimate adipose tissue dysfunction. Relation with obesity-associated cardiometabolic risk. Nutrients 2019;11: pii: E454.

Fruhbeck G, Catalán V, Rodríguez A, Gómez-Ambrosi J. Adiponectin-leptin ratio: a promising index to estimate adipose tissue dysfunction. Relation with obesity-associated cardiometabolic risk. Adipocyte. 2018 Jan 2;7(1):57-62.

Fujita T, Watanabe H, Murata Y, Hemmi S, Yabuki M, Fuke Y, Satomura A, Soma M. Plasma C1q/TNF-related protein 9: a promising biomarker for diabetic renal vascular injury. Minerva Urologica e Nefrologica= The Italian Journal of Urology and Nephrology. 2015 Dec 3;69(2):195-200.

Fujiyoshi A, Miura K, Kadowaki S, Azuma K, Tanaka S, Hisamatsu T, Arima H, Kadota A, Miyagawa N, Takashima N, Ohkubo T. Lifetime cigarette smoking is associated with abdominal obesity in a community-based sample of Japanese men: The Shiga Epidemiological Study of Subclinical Atherosclerosis (SESSA). Preventive medicine reports. 2016 Dec 1;4:225-32.

Gabir MM, Hanson RL, Dabelea D, Imperatore GI, Roumain JA, Bennett PH, Knowler WC. Plasma glucose and prediction of microvascular disease and mortality: evaluation of 1997 American Diabetes Association and 1999 World Health Organization criteria for diagnosis of diabetes. Diabetes care. 2000 Aug 1;23(8):1113-8.

Gahagan S. Overweight and obesity. Nelson's textbook of pediatrics, vol. 1, 21st ed. Philadelphia: Elsevier; 2020. p.1876—905.

Gavin III JR, Alberti KG, Davidson MB, DeFronzo RA. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes care. 1997 Jul 1;20(7):1183.

Gerstein HC, Santaguida P, Raina P, Morrison KM, Balion C, Hunt D, Yazdi H, Booker L. Annual incidence and relative risk of diabetes in people with various categories of dysglycemia: a systematic overview and meta-analysis of prospective studies. Diabetes research and clinical practice. 2007 Dec 1;78(3):305-12.

Ghadge AA, Khaire AA. Leptin as a predictive marker for metabolic syndrome. Cytokine 2019;121:154735.

Gokhale NH, Acharya AB, Patil VS, Trivedi DJ, Setty S, Thakur SL, *et al*.,. Resistin levels in gingival crevicular fluid of patients with chronic periodontitis and type 2 diabetes mellitus. J Periodontol. 2014;85:610–7.

Goldenberg R, Punthakee Z. Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. Canadian journal of diabetes. 2013 Apr 1;37:S8-11.

Groop LC, Bonadonna RC, DelPrato S, Ratheiser K, Zyck K, Ferrannini E, DeFronzo RA. Glucose and free fatty acid metabolism in non-insulin-dependent diabetes mellitus. Evidence for multiple sites of insulin resistance. The Journal of clinical investigation. 1989 Jul 1;84(1):205-13.

Gu HF. Biomarkers of adiponectin: plasma protein variation and genomic DNA polymorphisms. Biomarker insights. 2009 Jan;4:BMI-S3453.

Gujral UP, Narayan KV, Kahn SE, Kanaya AM. The relative associations of β-cell function and insulin sensitivity with glycemic status and incident glycemic progression in migrant Asian Indians in the United States: the MASALA study. Journal of Diabetes and its Complications. 2014 Jan 1;28(1):45-50.

Haffner SM. Insulin resistance, inflammation, and the prediabetic state. The American journal of cardiology. 2003 Aug 18;92(4):18-26.

Hajna S, White T, Brage S, van Sluijs EM, Westgate K, Jones AP, Luben R, Khaw KT, Wareham NJ, Griffin SJ. Descriptive epidemiology of changes in objectively measured sedentary behaviour and physical activity: six-year follow-up of the EPIC-Norfolk cohort. International Journal of Behavioral Nutrition and Physical Activity. 2018 Dec;15(1):1-9.

Hamman RF. Genetic and environmental determinants of non-insulin-dependent diabetes mellitus (NIDDM). Diabetes/metabolism reviews. 1992 Dec;8(4):287-338.

Hanley AJ, Wagenknecht LE, D'Agostino Jr RB, Zinman B, Haffner SM. Identification of subjects with insulin resistance and β-cell dysfunction using alternative definitions of the metabolic syndrome. Diabetes. 2003 Nov 1;52(11):2740-7.

Hansen D, Dendale P, Beelen M, Jonkers RA, Mullens A, Corluy L, Meeusen R, van Loon LJ. Plasma adipokine and inflammatory marker concentrations are altered in obese, as opposed to non-obese, type 2 diabetes patients. European journal of applied physiology. 2010 Jun;109(3):397-404.

Hara T, Fujiwara H, Shoji T, Mimura T, Nakao H, Fujimoto S. Decreased plasma adiponectin levels in young obese males. Journal of atherosclerosis and thrombosis. 2003;10(4):234-8.

Harjutsalo V, Forsblom C, Groop PH. Time trends in mortality in patients with type 1 diabetes: nationwide population based cohort study. Bmj. 2011 Sep 8;343.

Harrison TA, Hindorff LA, Kim H, Wines RC, Bowen DJ, McGrath BB, Edwards KL. Family history of diabetes as a potential public health tool. American journal of preventive medicine. 2003 Feb 1;24(2):152-9.

Heianza Y, Hara S, Arase Y, Saito K, Fujiwara K, Tsuji H, Kodama S, Hsieh SD, Mori Y, Shimano H, Yamada N. HbA1c 5· 7–6· 4% and impaired fasting plasma glucose for

diagnosis of prediabetes and risk of progression to diabetes in Japan (TOPICS 3): a longitudinal cohort study. The Lancet. 2011 Jul 9;378(9786):147-55.

Helmerhorst HJ, Wijndaele K, Brage S, Wareham NJ, Ekelund U. Objectively measured sedentary time may predict insulin resistance independent of moderate-and vigorous-intensity physical activity. Diabetes. 2009 Aug 1;58(8):1776-9.

Hemmingsen B, Gimenez-Perez G, Mauricio D, i Figuls MR, Metzendorf MI, Richter B. Diet, physical activity or both for prevention or delay of type 2 diabetes mellitus and its associated complications in people at increased risk of developing type 2 diabetes mellitus. Cochrane Database of Systematic Reviews. 2017(12).

Herder C, Schneitler S, Rathmann W, Haastert B, Schneitler H, Winkler H, Bredahl R, Hahnloser E, Martin S. Low-grade inflammation, obesity, and insulin resistance in adolescents. The Journal of Clinical Endocrinology & Metabolism. 2007 Dec 1;92(12):4569-74.

Herman WH, Edelstein SL, Ratner RE, Montez MG, Ackermann RT, Orchard TJ, Foulkes MA, Zhang P, Saudek CD, Brown MB, Diabetes Prevention Program Research Group. Effectiveness and cost-effectiveness of diabetes prevention among adherent participants. The American journal of managed care. 2013;19(3):194.

Herold KC, Bundy BN, Long SA, Bluestone JA, DiMeglio LA, Dufort MJ, Gitelman SE, Gottlieb PA, Krischer JP, Linsley PS, Marks JB. An anti-CD3 antibody, teplizumab, in relatives at risk for type 1 diabetes. New England Journal of Medicine. 2019 Aug 15;381(7):603-13.

Herron CA. Screening in diabetes mellitus: report of the Atlanta workshop. Diabetes care. 1979 Jul 1;2(4):357-62.

Hijjawi NS, Al-Radaideh AM, Al-Fayomi KI, Nimer NA, Alabadi HA, Al-Zu'bi RM, Agraib LM, Allehdan SS, Tayyem RF. Relationship of serum leptin with some biochemical, anthropometric parameters and abdominal fat volumes as measured by magnetic resonance imaging. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2018 May 1;12(3):207-13.

Hirani V, Ali A. BMI, overweight, and obesity (Chapter 5). In Craig R, Mindell J (eds.) Health Survey for England 2006. Volume 1. Cardiovascular disease and risk factors in adults.

Hoffstedt J, Eriksson P, Mottagui-Tabar S, Arner P. A polymorphism in the leptin promoter region (-2548 G/A) influences gene expression and adipose tissue secretion of leptin. Hormone and metabolic research. 2002 Jul;34(07):355-9.

Holman RR, Haffner SM, McMurray JJ, Bethel MA, Holzhauer B, Hua TA, Belenkov Y, Boolell M, Buse JB, Buckley BM, Chacra AR. Effect of Nateglinide on the Incidence of Diabetes and Cardiovascular Events.(vol 362, pg 1463, 2010). NEW ENGLAND JOURNAL OF MEDICINE. 2010 May 6;362(18):1748-.

Hoogwerf BJ, Sprecher DL, Pearce GL, Acevedo M, Frolkis JP, Foody JM, Cross JA, Pashkow FJ, Robinson K, Vidt DG. Blood glucose concentrations≤ 125 mg/dl and coronary heart disease risk. American Journal of Cardiology. 2002 Mar 1;89(5):596-9.

Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, Hinokio Y, Lindner TH, Mashima H, Schwarz PE, del Bosque-Plata L. Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. Nature genetics. 2000 Oct;26(2):163-75.

Hossain MM, Murali MR, Kamarul T. Genetically modified mesenchymal stem/stromal cells transfected with adiponectin gene can stably secrete adiponectin. Life sciences. 2017 Aug 1;182:50-6.

Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α-and obesity-induced insulin resistance. Science. 1996 Feb 2;271(5249):665-70.

Hsiao TJ, Lin E. A validation study of adiponectin rs266729 gene variant with type 2 diabetes, obesity, and metabolic phenotypes in a Taiwanese population. Biochemical genetics. 2016 Dec;54(6):830-41.

Hu FB, Van Dam RM, Liu S. Diet and risk of type II diabetes: the role of types of fat and carbohydrate. Diabetologia. 2001 Jul;44(7):805-17.

Hung WC, Wang CP, Lu LF, Yu TH, Chiu CA, Chung FM, Chen HJ, Houng JY, Shin SJ, Lee YJ. Circulating adiponectin level is associated with major adverse cardiovascular events in type 2 diabetic patients with coronary artery disease. Endocrine journal. 2010;57(9):793-802.

Huo L, Magliano DJ, Rancière F, Harding JL, Nanayakkara N, Shaw JE, Carstensen B. Impact of age at diagnosis and duration of type 2 diabetes on mortality in Australia 1997–2011. Diabetologia. 2018 May;61(5):1055-63.

Hur NW, Kim HC, Mo Nam C, Ha Jee S, Lee HC, Suh I. Smoking cessation and risk of type 2 diabetes mellitus: Korea Medical Insurance Corporation Study. European Journal of Preventive Cardiology. 2007 Apr 1;14(2):244-9.

International Diabetes Federation. IDF Diabetes Atlas, 6th ed. Brussels, Belgium: International Diabetes Federation; 2013.

Iwashima Y, Katsuya T, Ishikawa K, Ouchi N, Ohishi M, Sugimoto K, Fu Y, Motone M, Yamamoto K, Matsuo A, Ohashi K. Hypoadiponectinemia is an independent risk factor for hypertension. Hypertension. 2004 Jun 1;43(6):1318-23.

Jannasch F, Kröger J, Schulze MB. Dietary patterns and type 2 diabetes: a systematic literature review and meta-analysis of prospective studies. The Journal of nutrition. 2017 Jun 1;147(6):1174-82.

Jiang W, Tang Q, Zhang L, Chao Y, Hong B. Association of serum C1q tumour necrosis factor–related protein 9 with the severity of lower extremity peripheral arterial disease in type 2 diabetes patients. Diabetes and Vascular Disease Research. 2018 May;15(3):270-3.

Jung UJ, Choi MS. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. International journal of molecular sciences. 2014 Apr 11;15(4):6184-223.

Juraschek SP, Steffes MW, Selvin E. Associations of alternative markers of glycemia with hemoglobin A1c and fasting glucose. Clinical chemistry. 2012 Dec 1;58(12):1648-55.

Kadowaki T, Yamauchi T, Kubota N, *et al*.,. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 2006;116:1784–92.

Karjalainen J, Martin JM, Knip M, Ilonen J, Robinson BH, Savilahti E, Akerblom HK, Dosch HM. A bovine albumin peptide as a possible trigger of insulin-dependent diabetes mellitus. New England journal of medicine. 1992 Jul 30;327(5):302-7.

Karve A, Hayward RA. Prevalence, diagnosis, and treatment of impaired fasting glucose and impaired glucose tolerance in nondiabetic US adults. Diabetes care. 2010 Nov 1;33(11):2355-9.

Kasuga M. Insulin resistance and pancreatic β cell failure. The Journal of clinical investigation. 2006 Jul 3;116(7):1756-60.

Katikireddi SV, Morling JR, Bhopal R. Is there a divergence in time trends in the prevalence of impaired glucose tolerance and diabetes? A systematic review in South Asian populations. International journal of epidemiology. 2011 Dec 1;40(6):1542-53.

Kawamori R, Tajima N, Iwamoto Y, Kashiwagi A, Shimamoto K, Kaku K. 2009. Voglibose for prevention of type 2 diabetes mellitus: a randomised, double-blind trial in Japanese individuals with impaired glucose tolerance. Lancet 373(9675):1607–14.

Kazmi A, Tariq KM, Hashim R. Association of leptin with type 2 diabetes in non-obese subjects. Journal of Ayub Medical College Abbottabad. 2012 Dec 1;24(3-4):186-9.

Kendall DM, Cuddihy RM, Bergenstal RM. Clinical application of incretin-based therapy: therapeutic potential, patient selection and clinical use. The American journal of medicine. 2009 Jun 1;122(6):S37-50.

Khan RM, Chua ZJ, Tan JC, Yang Y, Liao Z, Zhao Y. From pre-diabetes to diabetes: diagnosis, treatments and translational research. Medicina. 2019 Aug 29;55(9):546.

Kharroubi I, Rasschaert J, Eizirik DL, Cnop M. Expression of adiponectin receptors in pancreatic β cells. Biochemical and biophysical research communications. 2003 Dec 26;312(4):1118-22.

Khaw K, Wareham N, Luben R. Glycated hemoglobin, diabetes and mortality in men in Norfolk cohort of european prospective investigation of cancer and nutrition.2001.

Khetan AK, Rajagopalan S. Prediabetes. Canadian Journal of Cardiology. 2018 May 1;34(5):615-23.

Kim K, Kim KH, Kim HH, Cheong J. Hepatitis B virus X protein induces lipogenic transcription factor SREBP1 and fatty acid synthase through the activation of nuclear receptor LXRα. Biochemical Journal. 2008 Dec 1;416(2):219-30.

Kim, W.K., Bae, K.H., Lee, S.C. and Oh, K.J., 2019. The latest insights into adipokines in diabetes. Journal of clinical medicine, 8(11), p.1874.

Kissebah AH, Sonnenberg GE, Myklebust J, Goldstein M, Broman K, James RG, Marks JA, Krakower GR, Jacob HJ, Weber J, Martin L. Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. Proceedings of the National Academy of Sciences. 2000 Dec 19;97(26):14478-83.

Klein, S., Allison, D.B., Heymsfield, S.B., Kelley, D.E., Leibel, R.L., Nonas, C. and Kahn, R., 2007. Waist circumference and cardiometabolic risk: a consensus statement from shaping America's health: Association for Weight Management and Obesity Prevention; NAASO, the Obesity Society; the American Society for Nutrition; and the American Diabetes Association. The American journal of clinical nutrition, 85(5), pp.1197-1202.

Knott C, Bell S, Britton A. Alcohol consumption and the risk of type 2 diabetes: a systematic review and dose-response meta-analysis of more than 1.9 million individuals from 38 observational studies. Diabetes care. 2015 Sep 1;38(9):1804-12.

Knowler WC, Fowler SE, Hamman RF, Christophi CA, Hoffman HJ, Brenneman AT, Brown-Friday JO, Goldberg R, Venditti E, Nathan DM. 10-year follow-up of diabetes incidence and weight loss in the Diabetes Prevention Program Outcomes Study. Lancet (London, England). 2009 Oct 29;374(9702):1677-86.

Ko GT, Chan JC, Tsang LW, Critchley JA, Cockram CS. Smoking and diabetes in Chinese men. Postgraduate medical journal. 2001 Apr 1;77(906):240-3.

Kocot J, Dziemidok P, Kiełczykowska M, Hordyjewska A, Szcześniak G, Musik I. Adipokine profile in patients with type 2 diabetes depends on degree of obesity. Medical science monitor: international medical journal of experimental and clinical research. 2017;23:4995.

Koloverou E, Esposito K, Giugliano D, Panagiotakos D. The effect of Mediterranean diet on the development of type 2 diabetes mellitus: a meta-analysis of 10 prospective studies and 136,846 participants. Metabolism. 2014 Jul 1;63(7):903-11.

Kramer CK, Zinman B, Retnakaran R. Short-term intensive insulin therapy in type 2 diabetes mellitus: a systematic review and meta-analysis. The lancet Diabetes & endocrinology. 2013 Sep 1;1(1):28-34.

Kubota N, Terauchi Y, Kubota T, Kumagai H, Itoh S, Satoh H, Yano W, Ogata H, Tokuyama K, Takamoto I, Mineyama T. Pioglitazone ameliorates insulin resistance and diabetes

by both adiponectin-dependent and-independent pathways. Journal of Biological Chemistry. 2006 Mar 31;281(13):8748-55.

Kunnari A, Ukkola O, Kesäniemi YA. Resistin polymorphisms are associated with cerebrovascular disease in Finnish Type 2 diabetic patients. Diabetic Medicine. 2005 May;22(5):583-9.

Kurihara O, Takano M, Yamamoto M, Shirakabe A, Kimata N, Inami T, Kobayashi N, Munakata R, Murakami D, Inami S, Okamatsu K. Impact of prediabetic status on coronary atherosclerosis: a multivessel angioscopic study. Diabetes care. 2013 Mar 1;36(3):729-33.

Kyrou I, Tsigos C, Mavrogianni C, Cardon G, Van Stappen V, Latomme J, Kivelä J, Wikström K, Tsochev K, Nanasi A, Semanova C. Sociodemographic and lifestyle-related risk factors for identifying vulnerable groups for type 2 diabetes: a narrative review with emphasis on data from Europe. BMC endocrine disorders. 2020 Mar;20(1):1-3.

Lai H, Lin N, Xing Z, Weng H, Zhang H. Association between the level of circulating adiponectin and prediabetes: A meta-analysis. Journal of diabetes investigation. 2015 Jul;6(4):416-29.

Laivuori H, Kaaja R, Koistinen H, Karonen SL, Andersson S, Koivisto V, Ylikorkala O. Leptin during and after preeclamptic or normal pregnancy: its relation to serum insulin and insulin sensitivity. Metabolism. 2000 Feb 1;49(2):259-63.

Lakka HM, Oksanen L, Tuomainen TP, Kontula K, Salonen JT. The common pentanucleotide polymorphism of the 3′-untranslated region of the leptin receptor gene is associated with serum insulin levels and the risk of type 2 diabetes in non-diabetic men: a prospective case–control study. Journal of internal medicine. 2000 Jul;248(1):77-83.

Lalrindiki C, Chanu CL, Shaini L, Nongtdu B, Sadanandam S, Devi WD, *et al*.,. Evaluation of Plasma Leptin in Type 2 Diabetes Mellitus. J Dent Med Sci. 2019;18(1):31–5.

Lamb MM, Miller M, Seifert JA, Frederiksen B, Kroehl M, Rewers M, Norris JM. The effect of childhood cow's milk intake and HLA-DR genotype on risk of islet autoimmunity and type 1 diabetes: The Diabetes Autoimmunity Study in the Young. Pediatric diabetes. 2015 Feb;16(1):31-8.

Lara-Castro C, Luo N, Wallace P, Klein RL, Garvey WT. Adiponectin multimeric complexes and the metabolic syndrome trait cluster. Diabetes. 2006 Jan 1;55(1):249-59.

Larsson SC, Permert J, Håkansson N, Näslund I, Bergkvist L, Wolk A. Overall obesity, abdominal adiposity, diabetes and cigarette smoking in relation to the risk of pancreatic cancer in two Swedish population-based cohorts. British journal of cancer. 2005 Nov;93(11):1310-5.

Lau CH, Muniandy S. Adiponectin and resistin gene polymorphisms in association with their respective adipokine levels. Annals of human genetics. 2011 May;75(3):370-82.

Lee MW, Lee M, Oh KJ. Adipose tissue-derived signatures for obesity and type 2 diabetes: adipokines, batokines and microRNAs. Journal of clinical medicine. 2019 Jun 14;8(6):854.

Lewitt MS, Hilding A, Brismar K, Efendic S, Östenson CG, Hall K. IGF-binding protein 1 and abdominal obesity in the development of type 2 diabetes in women. European journal of endocrinology. 2010 Aug 1;163(2):233-42.

Ley SH, Hamdy O, Mohan V, Hu FB. Prevention and management of type 2 diabetes: dietary components and nutritional strategies. The Lancet. 2014 Jun 7;383(9933):1999-2007.

Li Y, Teng DI, Shi X, Qin G, Qin Y, Quan H, Shi B, Sun H, Ba J, Chen B, Du J. Prevalence of diabetes recorded in mainland China using 2018 diagnostic criteria from the American Diabetes Association: national cross sectional study. bmj. 2020 Apr 28;369.

Liang, W. and dong Ye, D., 2019. The potential of adipokines as biomarkers and therapeutic agents for vascular complications in type 2 diabetes mellitus. Cytokine & growth factor reviews, 48, pp.32-39.

Lindström J, Peltonen M, Eriksson JG, Ilanne-Parikka P, Aunola S, Keinänen-Kiukaanniemi S, Uusitupa M, Tuomilehto J. Improved lifestyle and decreased diabetes risk over 13 years: long-term follow-up of the randomised Finnish Diabetes Prevention Study (DPS). Diabetologia. 2013 Feb;56(2):284-93.

Lister R, Pelizzola M, Dowen RH, Hawkins RD, Hon G, Tonti-Filippini J, Nery JR, Lee L, Ye Z, Ngo QM, Edsall L. Human DNA methylomes at base resolution show widespread epigenomic differences. nature. 2009 Nov;462(7271):315-22.

Liu J, Wu YY, Huang XM, Yang M, Zha BB, Wang F, Zha Y, Sheng L, Chen ZP, Gu Y. Ageing and type 2 diabetes in an elderly Chinese population: the role of insulin resistance and beta cell dysfunction. Eur Rev Med Pharmacol Sci. 2014 Jan 1;18(12):1790-7.

Lönnrot M, Lynch KF, Elding Larsson H, Lernmark Å, Rewers MJ, Törn C, Burkhardt BR, Briese T, Hagopian WA, She JX, Simell OG. Respiratory infections are temporally associated with initiation of type 1 diabetes autoimmunity: the TEDDY study. Diabetologia. 2017 Oct;60(10):1931-40.

Lorenzo C, Wagenknecht LE, Hanley AJ, Rewers MJ, Karter AJ, Haffner SM. A1C between 5.7 and 6.4% as a marker for identifying pre-diabetes, insulin sensitivity and secretion, and cardiovascular risk factors: the Insulin Resistance Atherosclerosis Study (IRAS). Diabetes care. 2010 Sep 1;33(9):2104-9.

Mabrouk R, Ghareeb H, Shehab A, Omar K, El-Kabarity RH, Soliman DA, Mohamed NA. Serum visfatin, resistin and IL-18 in A group of Egyptian obese diabetic and non diabetic individuals. Egypt J Immunol. 2013 Jan 1;20(1):1-1.

Madsbad S, McNair P, Christensen MS, Christiansen C, Faber OK, Binder C, Transbøl I. Influence of smoking on insulin requirement and metbolic status in diabetes mellitus. Diabetes care. 1980 Jan 1;3(1):41-3.

Maffei Á, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, Kern PA. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nature medicine. 1995 Nov;1(11):1155-61.

Maffeis C, Pinelli L, Brambilla P, Banzato C, Valzolgher L, Ulmi D, Di Candia S, Cammarata B, Morandi A. Fasting plasma glucose (FPG) and the risk of impaired glucose tolerance in obese children and adolescents. Obesity. 2010 Jul;18(7):1437-42.

Mann DM, Carson AP, Shimbo D, Fonseca V, Fox CS, Muntner P. Impact of A1C screening criterion on the diagnosis of pre-diabetes among US adults. Diabetes Care. 2010 Oct 1;33(10):2190-5.

Mantzoros CS, Moschos SJ. Leptin: in search of role (s) in human physiology and pathophysiology. Clinical endocrinology. 1998 Nov;49(5):551-67.

Marcovecchio ML, Lucantoni M, Chiarelli F. Role of chronic and acute hyperglycemia in the development of diabetes complications. Diabetes technology & therapeutics. 2011 Mar 1;13(3):389-94.

Mazahreh TS, Alfaqih M, Saadeh R, Al-Zoubi NA, Hatamleh M, Alqudah A, Aleshawi AJ, Alzoubi A. The effects of laparoscopic sleeve gastrectomy on the parameters of leptin resistance in obesity. Biomolecules. 2019 Sep 25;9(10):533.

McTernan PG, Kusminski CM, Kumar S. Resistin. Current opinion in lipidology. 2006 Apr 1;17(2):170-5.

Medina-Bravo P, Meza-Santibáñez R, Rosas-Fernández P, Galván-Duarte R, Saucedo-García R, Velázquez-López L, Torres-Tamayo M. Decrease in serum adiponectin levels associated with visceral fat accumulation independent of pubertal stage in children and adolescents. Archives of medical research. 2011 Feb 1;42(2):115-21.

Melidonis A, Tournis S, Kompoti MG, Lentzas I, Roussou V, Iraklianou S, Michail I, Mariolis A. Increased prevalence of diabetes mellitus in a rural Greek population. Rural and Remote Health. 2006 Mar 1;6(1):1-8.

Menzaghi C, Trischitta V, Doria A. Genetic influences of adiponectin on insulin resistance, type 2 diabetes, and cardiovascular disease. Diabetes. 2007 May 1;56(5):1198-209.

Meshkani R, Taheripak A, Zarghooni G, Rezaei M, Sadeghi M, Eshkiki AZ. Association between Leptin G2548A and leptin receptor Q223R polymorphisms with type 2 diabetes in an iranian population. Clin. Lab. 2016 Jan 1;62(1-2):89-96.

Mijajlović MD, Aleksić VM, Šternić NM, Mirković MM, Bornstein NM. Role of prediabetes in stroke. Neuropsychiatric disease and treatment. 2017;13:259.

Mihai BM, Petriş AO, Ungureanu DA, Lăcătuşu CM. Insulin resistance and adipokine levels correlate with early atherosclerosis–a study in prediabetic patients. Open Medicine. 2014 Sep 26;10(1).

Misra A, Shrivastava U. Obesity and dyslipidemia in South Asians. Nutrients. 2013 Jul 16;5(7):2708-33.

Misra A, Singhal N, Sivakumar B, Bhagat N, Jaiswal A, Khurana L. Nutrition transition in India: Secular trends in dietary intake and their relationship to diet-related non-communicable diseases. Journal of diabetes. 2011 Dec;3(4):278-92.

Miyamoto Y, Morisaki H, Kokubo Y, Yamanaka I, Tomoike H, Okayama A, Yoshimasa Y, Morisaki T. Resistin gene variations are associated with the metabolic syndrome in Japanese men. Obesity Research & Clinical Practice. 2009 May 1;3(2):65-74.

Moebus S, Stang A, Möhlenkamp S, Dragano N, Schmermund A, Slomiany U, Hoffmann B, Bauer M, Broecker-Preuss M, Mann K, Siegrist J. Association of impaired fasting glucose and coronary artery calcification as a marker of subclinical atherosclerosis in a population-based cohort—results of the Heinz Nixdorf Recall Study. Diabetologia. 2009 Jan;52(1):81-9.

Mohammadzadeh G, Ghaffari MA, Heibar H, Bazyar M. Association of two common single nucleotide polymorphisms (+ 45T/G and+ 276G/T) of ADIPOQ gene with coronary artery disease in type 2 diabetic patients. Iranian biomedical journal. 2016 Jul;20(3):152.

Mohan V, Amutha A, Ranjani H, Unnikrishnan R, Datta M, Anjana RM, Staimez L, Ali MK, Narayan KV. Associations of β-cell function and insulin resistance with youth-onset type 2 diabetes and prediabetes among Asian Indians. Diabetes technology & therapeutics. 2013 Apr 1;15(4):315-22.

Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group*. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Annals of internal medicine. 2009 Aug 18;151(4):264-9.

Mori Y, Otabe S, Dina C, Yasuda K, Populaire C, Lecoeur C, Vatin V, Durand E, Hara K, Okada T, Tobe K. Genome-wide search for type 2 diabetes in Japanese affected sib-pairs confirms susceptibility genes on 3q, 15q, and 20q and identifies two new candidate Loci on 7p and 11p. Diabetes. 2002 Apr 1;51(4):1247-55.

Morimoto A, Tatsumi Y, Deura K, Mizuno S, Ohno Y, Miyamatsu N, Watanabe S. Impact of impaired insulin secretion and insulin resistance on the incidence of type 2 diabetes mellitus in a Japanese population: the Saku study. Diabetologia. 2013 Aug;56(8):1671-9.

Moustgaard H, Bjerregaard P, Borch-Johnsen K, Jørgensen ME, The Greenland Population Study. Diabetes among Inuit migrants in Denmark. International Journal of Circumpolar Health. 2005 Sep 1;64(4):354-64.

Murea M, Ma L, Freedman BI. Genetic and environmental factors associated with type 2 diabetes and diabetic vascular complications. The review of diabetic studies: RDS. 2012;9(1):6.

Nagaev I, Smith U. Insulin resistance and type 2 diabetes are not related to resistin expression in human fat cells or skeletal muscle. Biochemical and biophysical research communications. 2001 Jul 13;285(2):561-4.

Nakano Y, Tobe T, Choi-Miura NH, Mazda T, Tomita M. Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. The Journal of Biochemistry. 1996 Oct 1;120(4):803-12.

Narendran P. Screening for type 1 diabetes: are we nearly there yet?. Diabetologia. 2019 Jan;62(1):24-7.

Nasri H, Rafieian-Kopaei M. Diabetes mellitus and renal failure: Prevention and management. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences. 2015 Nov;20(11):1112.

Nathan DM, Davidson MB, DeFronzo RA, Heine RJ, Henry RR, Pratley R, Zinman B. Impaired fasting glucose and impaired glucose tolerance: implications for care. Diabetes care. 2007 Mar 1;30(3):753-9.

Neamah HH, Kuhlmann AK, Tabak RG. Effectiveness of program modification strategies of the diabetes prevention program: a systematic review. The Diabetes Educator. 2016 Apr;42(2):153-65.

Nebuchennykh M, Løseth S, Jorde R, Mellgren SI. Idiopathic polyneuropathy and impaired glucose metabolism in a Norwegian patient series. European journal of neurology. 2008 Aug;15(8):810-6.

Neuparth MJ, Proença JB, Santos-Silva A, Coimbra S: Adipokines, oxidized low-density lipoprotein, and C-reactive protein levels in lean, overweight, and obese portuguese patients with type 2 diabetes. ISRN Obes, 2013; 2013: 142097.

Nichols GA, Hillier TA, Brown JB. Progression from newly acquired impaired fasting glusose to type 2 diabetes. Diabetes care. 2007 Feb 1;30(2):228-33.

Nogueiras R, Pérez-Tilve D, Veyrat-Durebex C, Morgan DA, Varela L, Haynes WG, Patterson JT, Disse E, Pfluger PT, López M, Woods SC. Direct control of peripheral lipid deposition by CNS GLP-1 receptor signaling is mediated by the sympathetic nervous system and blunted

in diet-induced obesity. Journal of Neuroscience. 2009 May 6;29(18):5916-25.G, Zhang P, Wang J, Gregg EW, Yang W, Gong Q, Li H, Li H, Jiang Y, An Y, Shuai Y. The long-term effect of lifestyle interventions to prevent diabetes in the China Da Qing Diabetes Prevention Study: a 20-year follow-up study. The Lancet. 2008 May 24;371(9626):1783-9.

Nogueiras R, Pérez-Tilve D, Veyrat-Durebex C, Morgan DA, Varela L, Haynes WG, Patterson JT, Disse E, Pfluger PT, López M, Woods SC. Direct control of peripheral lipid deposition by CNS GLP-1 receptor signaling is mediated by the sympathetic nervous system and blunted in diet-induced obesity. Journal of Neuroscience. 2009 May 6;29(18):5916-25.

Norata GD, Ongari M, Garlaschelli K, Raselli S, Grigore L, Catapano AL. Plasma resistin levels correlate with determinants of the metabolic syndrome. European journal of endocrinology. 2007 Feb 1;156(2):279-84.

Nyamdorj R, Pitkäniemi J, Tuomilehto J, Hammar N, Stehouwer CD, Lam TH, Ramachandran A, Janus ED, Mohan V, Söderberg S, Laatikainen T. Ethnic comparison of the association of undiagnosed diabetes with obesity. International journal of obesity. 2010 Feb;34(2):332-9.

Oh KJ, Lee DS, Kim WK, Han BS, Lee SC, Bae KH. Metabolic adaptation in obesity and type II diabetes: myokines, adipokines and hepatokines. International journal of molecular sciences. 2016 Dec 22;18(1):8.

Ohmori R, Momiyama Y, Kato R, Taniguchi H, Ogura M, Ayaori M, Nakamura H, Ohsuzu F. Associations between serum resistin levels and insulin resistance, inflammation, and coronary artery disease. Journal of the American College of Cardiology. 2005 Jul 19;46(2):379-80.

Okereke OI, Kurth T, Pollak MN, Gaziano JM, Grodstein F. Fasting plasma insulin, C-peptide and cognitive change in older men without diabetes: results from the Physicians' Health Study II. Neuroepidemiology. 2010;34(4):200-7.

Onuma H, Tabara Y, Kawamura R, Tanaka T, Ohashi J, Nishida W, Takata Y, Ochi M, Yamada K, Kawamoto R, Kohara K. A at single nucleotide polymorphism-358 is required for G at-420 to confer the highest plasma resistin in the general Japanese population. PloS one. 2010 Mar 16;5(3):e9718.

Oral EA, Simha V, Ruiz E, Andewelt A, Premkumar A, Snell P, Wagner AJ, DePaoli AM, Reitman ML, Taylor SI, Gorden P. Leptin-replacement therapy for lipodystrophy. New England Journal of Medicine. 2002 Feb 21;346(8):570-8.

Osawa H, Tabara Y, Kawamoto R, Ohashi J, Ochi M, Onuma H, Nishida W, Yamada K, Nakura J, Kohara K, Miki T. Plasma resistin, associated with single nucleotide polymorphism− 420, is correlated with insulin resistance, lower HDL cholesterol, and high-sensitivity C-reactive protein in the Japanese general population. Diabetes care. 2007 Jun 1;30(6):1501-6.

Otero M, Lago R, Lago F, Casanueva FF, Dieguez C, Gomez-Reino JJ, Gualillo O: Leptin, from fat to inflammation: Old questions and new insights. FEBS Lett. 2005, 579: 295-301.

Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y, Ishigami M, Kuriyama H, Kishida K, Nishizawa H, Hotta K. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. Circulation. 2001 Feb 27;103(8):1057-63.

Pajvani UB, Scherer PE. Adiponectin: systemic contributor to insulin sensitivity. Current diabetes reports. 2003 May;3(3):207-13.

Palit SP, Patel R, Parmar N, Rathwa N, Dalvi N, Ramachandran AV, Begum R. Repurposing statin and L-glutamine: replenishing β-cells in hyperlipidemic T2D mouse model. bioRxiv. 2021 Jan 1.

Pan H, Guo J, Su Z. Advances in understanding the interrelations between leptin resistance and obesity. Physiology & behavior. 2014 May 10;130:157-69.

Park JW, Park J, Jee SH. ADIPOQ gene variants associated with susceptibility to obesity and low serum adiponectin levels in healthy Koreans. Epidemiology and health. 2011;33.

Park KS. The search for genetic risk factors of type 2 diabetes mellitus. Diabetes & metabolism journal. 2011 Feb 28;35(1):12-22.

Patel L, Buckels AC, Kinghorn IJ, Murdock PR, Holbrook JD, Plumpton C, Macphee CH, Smith SA. Resistin is expressed in human macrophages and directly regulated by PPARγ activators. Biochemical and biophysical research communications. 2003 Jan 10;300(2):472-6.

Path G. Bornstein SR, Gurniak M, Chrousos GP, Scherbaum WA, and Hauner H. Human breast adipocytes express interleukin-6 (IL-6) and its receptor system: increased IL-6 production by beta-adrenergic activation and effects of IL-6 on adipocyte function. J Clin Endocrinol Metab. 2001;86:2281-8.

Patja K, Jousilahti P, Hu G, Valle T, Qiao Q, Tuomilehto J. Effects of smoking, obesity and physical activity on the risk of type 2 diabetes in middle-aged Finnish men and women. Journal of internal medicine. 2005 Oct;258(4):356-62.

Patterson CC, Dahlquist G, Harjutsalo V, Joner G, Feltbower RG, Svensson J, Schober E, Gyürüs E, Castell C, Urbonaité B, Rosenbauer J. Early mortality in EURODIAB population-based cohorts of type 1 diabetes diagnosed in childhood since 1989. Diabetologia. 2007 Dec;50(12):2439-42.

Peng LN, Lin MH, Lai HY, Hwang SJ, Chen LK, Chiou ST. Risk factors of new onset diabetes mellitus among elderly Chinese in rural Taiwan. Age and ageing. 2010 Jan 1;39(1):125-8.

Peters SA, Huxley RR, Woodward M. Diabetes as risk factor for incident coronary heart disease in women compared with men: a systematic review and meta-analysis of 64 cohorts including 858,507 individuals and 28,203 coronary events. Diabetologia. 2014 Aug;57(8):1542-51.

Phillips LS, Ratner RE, Buse JB, Kahn SE. We can change the natural history of type 2 diabetes. Diabetes care. 2014 Oct 1;37(10):2668-76.

Picu A, Petcu L, Ştefan S *et al*.,: Markers of oxidative stress and antioxidant defense in romanian patients with type 2 diabetes mellitus and obesity. Molecules, 2017; 22(5): E714.

Pilz S, Horejsi R, M ller R, Almer G, Scharnagl H, Stojakovic T, Dimitrova R, Weihrauch G, Borkenstein M, Maerz W, Schauenstein K. Early atherosclerosis in obese juveniles is associated with low serum levels of adiponectin. The Journal of Clinical Endocrinology & Metabolism. 2005 Aug 1;90(8):4792-6.

Pirie K, Peto R, Reeves GK, Green J, Beral V, Million Women Study Collaborators. The 21st century hazards of smoking and benefits of stopping: a prospective study of one million women in the UK. The Lancet. 2013 Jan 12;381(9861):133-41.

Plantinga LC, Crews DC, Coresh J, Miller ER, Saran R, Yee J, Hedgeman E, Pavkov M, Eberhardt MS, Williams DE, Powe NR. Prevalence of chronic kidney disease in US adults with undiagnosed diabetes or prediabetes. Clinical Journal of the American Society of Nephrology. 2010 Apr 1;5(4):673-82.

Pramanik S, Rathwa N, Patel R, Ramachandran AV, Begum R. Treatment avenues for type 2 diabetes and current perspectives on adipokines. Current diabetes reviews. 2018 Jun 1;14(3):201-21.

Prior SL, Gable DR, Cooper JA, Bain SC, Hurel SJ, Humphries SE, Stephens JW. Association between the adiponectin promoter rs266729 gene variant and oxidative stress in patients with diabetes mellitus. European heart journal. 2009 May 1;30(10):1263-9.

Purkayastha S, Zhang G, Cai D. Uncoupling the mechanisms of obesity and hypertension by targeting hypothalamic IKK-β and NF-κB. Nature medicine. 2011 Jul;17(7):883-7.

Putz Z, Tabák ÁG, Tóth N, Istenes I, Nemeth N, Gandhi RA, Hermányi Z, Keresztes K, Jermendy G, Tesfaye S, Kempler P. Noninvasive evaluation of neural impairment in subjects with impaired glucose tolerance. Diabetes Care. 2009 Jan 1;32(1):181-3.

Qasim AN, Metkus TS, Tadesse M, Lehrke M, Restine S, Wolfe ML, Hannenhalli S, Cappola T, Rader DJ, Reilly MP. Resistin gene variation is associated with systemic inflammation but not plasma adipokine levels, metabolic syndrome or coronary atherosclerosis in nondiabetic Caucasians. Clinical endocrinology. 2009 May;70(5):698-705.

Qian L, Xu L, Wang X, Fu X, Gu Y, Lin F, Peng Y, Li G, Luo M. Early insulin secretion failure leads to diabetes in Chinese subjects with impaired glucose regulation. Diabetes/metabolism research and reviews. 2009 Feb;25(2):144-9.

Qin L, Xu H. A cross-sectional study of the effect of health literacy on diabetes prevention and control among elderly individuals with prediabetes in rural China. BMJ open. 2016 May 1;6(5):e011077.

Raghushaker CR, Shashank T, Manista A *et al*.,: Evaluation of serum bio markers [Leptin, Adiponectin, Crp, Oxidised- Ldl] and lipid parameter in obese with type-2 diabetes mellitus: Risk to CVD. JARBS, 2013; 5(3): 250–53.

Rajala MW, Obici S, Scherer PE, Rossetti L. Adipose-derived resistin and gut-derived resistin-like molecule–β selectively impair insulin action on glucose production. The Journal of clinical investigation. 2003 Jan 15;111(2):225-30.

Rajković N, Zamaklar M, Lalic K *et al.*,: Relationship between obesity, adipocytokines and inflammatory markers in type 2 diabetes: Relevance for cardiovascular risk prevention. Int J Environ Res Public Health, 2014; 11(4): 4049–65.

Rajmangal Choudhary Suryakant Nagtilak, S K Shukla. Association of adiponectin & leptin with insulin resistance in type-2 diabetes., International Journal of Clinical Biochemistry and Research 2020;7(4):446–450.

Ramachandran A, Snehalatha C, Mary S, Mukesh B, Bhaskar AD, Vijay V. The Indian Diabetes Prevention Programme shows that lifestyle modification and metformin prevent type 2 diabetes in Asian Indian subjects with impaired glucose tolerance (IDPP-1). Diabetologia. 2006 Feb;49(2):289-97.

Rao Kondapally Seshasai S, Kaptoge S, Thompson A, Di Angelantonio E, Gao P, Sarwar N, Whincup PH, Mukamal KJ, Gillum RF, Holme I, Njølstad I. Diabetes mellitus, fasting glucose, and risk of cause-specific death. The New England journal of medicine. 2011;364(9):829-41.

Rasmussen SS, Glümer C, Sandbaek A, Lauritzen T, Borch-Johnsen K. Determinants of progression from impaired fasting glucose and impaired glucose tolerance to diabetes in a high-risk screened population: 3 year follow-up in the ADDITION study, Denmark. Diabetologia. 2008 Feb;51(2):249-57.

Rathwa N, Patel R, Palit SP, Ramachandran AV, Begum R. Genetic variants of resistin and its plasma levels: Association with obesity and dyslipidemia related to type 2 diabetes susceptibility. Genomics. 2019 Jul 1;111(4):980-5.

Rathwa N., N. Patel, A.V. Ramchandran, R. Begum, Association of Resistin genetic variants with type II diabetes, Poster Presented at International Conference on Reproductive Biology and Comparative Endocrinology (ICRBCE) and The 35th Annual Meeting of the Society for Reproductive Biology and Comparative Endocrinology (SRBCE-XXXV), 2017 Feb, pp. 9–11 (Hyderabad, India).

Rawshani A, Sattar N, Franzén S, Rawshani A, Hattersley AT, Svensson AM, Eliasson B, Gudbjörnsdottir S. Excess mortality and cardiovascular disease in young adults with

type 1 diabetes in relation to age at onset: a nationwide, register-based cohort study. The Lancet. 2018 Aug 11;392(10146):477-86.

Reaven GM. Insulin resistance, the insulin resistance syndrome, and cardiovascular disease. Panminerva medica. 2005 Dec 1;47(4):201-10.

Reilly MP, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA, Rader DJ. Resistin is an inflammatory marker of atherosclerosis in humans. Circulation. 2005 Feb 22;111(7):932-9.

Rijkelijkhuizen JM, Nijpels G, Heine RJ, Bouter LM, Stehouwer CD, Dekker JM. High risk of cardiovascular mortality in individuals with impaired fasting glucose is explained by conversion to diabetes: the Hoorn study. Diabetes Care. 2007 Feb 1;30(2):332-6.

Rizza S, Cardellini M, Porzio O, Pecchioli C, Savo A, Cardolini I, Senese N, Lauro D, Sbraccia P, Lauro R, Federici M. Pioglitazone improves endothelial and adipose tissue dysfunction in pre-diabetic CAD subjects. Atherosclerosis. 2011 Mar 1;215(1):180-3.

Rode PA, Kolte RA, Kolte AP, Purohit HJ, Ahuja CR. Relevance of single-nucleotide polymorphism to the expression of resistin gene affecting serum and gingival crevicular fluid resistin levels in chronic periodontitis and type 2 diabetes mellitus: A randomized control clinical trial. Journal of Indian Society of Periodontology. 2019 Mar;23(2):131.

Rossetti L, Massillon D, Barzilai N, Vuguin P, Chen W, Hawkins M, Wu J, Wang J. Short term effects of leptin on hepatic gluconeogenesis and in vivo insulin action. Journal of Biological Chemistry. 1997 Oct 31;272(44):27758-63.

Roszkowska-Gancarz M, Kurylowicz A, Polosak J, Mossakowska M, Franek E, Puzianowska-Kuźnicka M. Functional polymorphisms of the leptin and leptin receptor genes are associated with longevity and with the risk of myocardial infarction and of type 2 diabetes mellitus. Endokrynologia Polska. 2014;65(1):11-6.

Rubino A, McQuay LJ, Gough SC, Kvasz M, Tennis P. Delayed initiation of subcutaneous insulin therapy after failure of oral glucose-lowering agents in patients with type 2 diabetes: a population-based analysis in the UK. Diabetic Medicine. 2007 Dec;24(12):1412-8.

Sabi EM, Bin Dahman LS, Mohammed AK, Sumaily KM, Al-Daghri NM. -2548G> A LEP Polymorphism Is Positively Associated with Increased Leptin and Glucose Levels in Obese Saudi Patients Irrespective of Blood Pressure Status. Medicina. 2022 Feb 24;58(3):346.

Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, Colagiuri S, Guariguata L, Motala AA, Ogurtsova K, Shaw JE. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. Diabetes research and clinical practice. 2019 Nov 1;157:107843.

Saito T, Watanabe M, Nishida J, Izumi T, Omura M, Takagi T, Fukunaga R, Bandai Y, Tajima N, Nakamura Y, Ito M. Lifestyle modification and prevention of type 2 diabetes in overweight Japanese with impaired fasting glucose levels: a randomized controlled trial. Archives of internal medicine. 2011 Aug 8;171(15):1352-60.

Salas-Salvadó J, Bulló M, Babio N, Martínez-González MÁ, Ibarrola-Jurado N, Basora J, Estruch R, Covas MI, Corella D, Arós F, Ruiz-Gutiérrez V. Reduction in the incidence of type 2 diabetes with the Mediterranean diet: results of the PREDIMED-Reus nutrition intervention randomized trial. Diabetes care. 2011 Jan 1;34(1):14-9.

Saltevo J, Laakso M, Jokelainen J, Keinänen-Kiukaanniemi S, Kumpusalo E, Vanhala M. Levels of adiponectin, C-reactive protein and interleukin-1 receptor antagonist are associated with insulin sensitivity: a population-based study. Diabetes/metabolism research and reviews. 2008 Jul;24(5):378-83.

Santilli F, Liani R, Di Fulvio P, Formoso G, Simeone P, Tripaldi R, Ueland T, Aukrust P, Davi G. Increased circulating resistin is associated with insulin resistance, oxidative stress and platelet activation in type 2 diabetes mellitus. Thrombosis and haemostasis. 2016;116(12):1089-99.

Sattar N, Rawshani A, Franzén S, Rawshani A, Svensson AM, Rosengren A, McGuire DK, Eliasson B, Gudbjörnsdottir S. Age at diagnosis of type 2 diabetes mellitus and associations with cardiovascular and mortality risks: findings from the Swedish National Diabetes Registry. Circulation. 2019 May 7;139(19):2228-37.

Schäffler A, Neumeier M, Herfarth H, Fürst A, Schölmerich J, Büchler C. Genomic structure of human omentin, a new adipocytokine expressed in omental adipose tissue.

Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression. 2005 Dec 30;1732(1-3):96-102.

Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. Journal of Biological chemistry. 1995 Nov 10;270(45):26746-9.

Schober AK, Neurath MF, Harsch IA. Prevalence of sleep apnoea in diabetic patients. The clinical respiratory journal. 2011 Jul;5(3):165-72.

Schulze MB, Manson JE, Ludwig DS, Colditz GA, Stampfer MJ, Willett WC, Hu FB. Sugar-sweetened beverages, weight gain, and incidence of type 2 diabetes in young and middle-aged women. Jama. 2004 Aug 25;292(8):927-34.

Schwartz DR, Lazar MA. Human resistin: found in translation from mouse to man. Trends in Endocrinology & Metabolism. 2011 Jul 1;22(7):259-65.

Selvin E, Steffes MW, Zhu H, Matsushita K, Wagenknecht L, Pankow J, Coresh J, Brancati FL. Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. New England Journal of Medicine. 2010 Mar 4;362(9):800-11.

Shahim B, De Bacquer D, De Backer G, Gyberg V, Kotseva K, Mellbin L, Schnell O, Tuomilehto J, Wood D, Rydén L. The prognostic value of fasting plasma glucose, two-hour postload glucose, and HbA1c in patients with coronary artery disease: a report from EUROASPIRE IV: a survey from the European Society of Cardiology. Diabetes Care. 2017 Sep 1;40(9):1233-40.

Shaw JE, Zimmet PZ, Hodge AM, De Courten M, Dowse GK, Chitson P, Tuomilehto J, Alberti KG. Impaired fasting glucose: how low should it go?. Diabetes Care. 2000 Jan 1;23(1):34-9.

Shen J, Kondal D, Rubinstein A, Irazola V, Gutierrez L, Miranda JJ, Bernabé-Ortiz A, Lazo-Porras M, Levitt N, Steyn K, Bobrow K. A multiethnic study of pre-diabetes and diabetes in LMIC. Global heart. 2016 Mar 1;11(1):61-70.

Shibata R, Murohara T, Ouchi N. Protective role of adiponectin in cardiovascular disease. Current medicinal chemistry. 2012 Nov 1;19(32):5459-66.

Silha JV, Krsek M, Skrha JV, Sucharda P, Nyomba BL, Murphy LJ. Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. European journal of endocrinology. 2003 Oct;149(4):331-5.

Skurk T, Alberti-Huber C, Herder C, Hauner H. Relationship between adipocyte size and adipokine expression and secretion. The Journal of Clinical Endocrinology & Metabolism. 2007 Mar 1;92(3):1023-33.

Sorkin JD, Muller DC, Fleg JL, Andres R. The relation of fasting and 2-h postchallenge plasma glucose concentrations to mortality: data from the Baltimore Longitudinal Study of Aging with a critical review of the literature. Diabetes care. 2005 Nov 1;28(11):2626-32.

Spranger J, Kroke A, Möhlig M, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF. Adiponectin and protection against type 2 diabetes mellitus. The Lancet. 2003 Jan 18;361(9353):226-8.

Stamler J, Vaccaro O, Neaton JD, Wentworth D, Multiple Risk Factor Intervention Trial Research Group. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. Diabetes care. 1993 Feb 1;16(2):434-44.

Stephens JM, Lee J, Pilch PF. Tumor necrosis factor-α-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without a loss of insulin receptor-mediated signal transduction. Journal of Biological Chemistry. 1997 Jan 10;272(2):971-6.

Stojanovic S, Deljanin-Ilić M, Ilić S, Petrović D, Đukić S. The significance of adiponectin as a biomarker in metabolic syndrome and/or coronary artery disease. Vojnosanitetski pregled. 2015;72(9):779-84.

Suastika K, Dwipayana P, Saraswati IM, Kuswardhani T, Astika N, Putrawan IB, Matsumoto K, Kajiwara N, Taniguchi H. Relationship between age and metabolic disorders in the population of Bali. Journal of Clinical Gerontology and Geriatrics. 2011 Jun 1;2(2):47-52.

Sumner CJ, Sheth S, Griffin JW, Cornblath DR, Polydefkis M. The spectrum of neuropathy in diabetes and impaired glucose tolerance. Neurology. 2003 Jan 14;60(1):108-11.

Sun Q, van Dam RM, Meigs JB, Franco OH, Mantzoros CS, Hu FB. Leptin and soluble leptin receptor levels in plasma and risk of type 2 diabetes in US women: a prospective study. Diabetes. 2010 Mar 1;59(3):611-8.

Suriyaprom K, Tungtrongchitr R, Namjuntra P. Associations of resistin levels with resistin gene polymorphism and metabolic syndrome in Thais. Journal of Medical Biochemistry. 2015 Apr;34(2):170.

Susilowati R, Sulistyoningrum DC, Witari NP, Huriyati E, Luglio HF, Julia M. Sexual dimorphism in interleukin 17A and adipocytokines and their association with insulin resistance among obese adolescents in Yogyakarta, Indonesia. Asia Pacific Journal of Clinical Nutrition. 2016 Dec 1;25(Suppl 1):S93-101.

Suzuki MM, Bird A. DNA methylation landscapes: provocative insights from epigenomics. Nature reviews genetics. 2008 Jun;9(6):465-76.

Tabák A.G., Herder C., Rathmann W., Brunner E.J., Kivimäki M. Prediabetes: A high-risk state for diabetes development. Lancet. 2012;379:2279–2290. doi: 10.1016/S0140-6736(12)60283-9.

Takahashi M, Arita Y, Yamagata K, Matsukawa Y, Okutomi K, Horie M, Shimomura I, Hotta K, Kuriyama H, Kihara S, Nakamura T. Genomic structure and mutations in adipose-specific gene, adiponectin. International journal of obesity. 2000 Jul;24(7):861-8.

Tan MS, Chang SY, Chang DM, Tsai JC, Lee YJ. Association of resistin gene 3′-untranslated region+ 62G→ A polymorphism with type 2 diabetes and hypertension in a Chinese population. The Journal of Clinical Endocrinology & Metabolism. 2003 Mar 1;88(3):1258-63.

Tang NP, Wang LS, Yang L, Zhou B, Gu HJ, Sun QM, Cong RH, Zhu HJ, Wang B. A polymorphism in the resistin gene promoter and the risk of coronary artery disease in a Chinese population. Clinical endocrinology. 2008 Jan;68(1):82-7.

Tatti P, Masselli L, Buonanno A, *et al*.,. Leptin levels in diabetic and nondiabetic subjects. Endocrine 2001;15:305–8.

Tesfaye S, Boulton AJ, Dyck PJ, Freeman R, Horowitz M, Kempler P, Lauria G, Malik RA, Spallone V, Vinik A, Bernardi L. Diabetic neuropathies: Update on definitions, diagnostic criteria, estimation of severity, and treatments (Diabetes Care (2010) 33,(2285-2293)). Diabetes Care. 2010 Dec;33(12):2725.

Tesfaye S, Chaturvedi N, Eaton SE, Ward JD, Manes C, Ionescu-Tirgoviste C, Witte DR, Fuller JH. Vascular risk factors and diabetic neuropathy. New England Journal of Medicine. 2005 Jan 27;352(4):341-50.

Thomas G, Sehgal AR, Kashyap SR, Srinivas TR, Kirwan JP, Navaneethan SD. Metabolic syndrome and kidney disease: a systematic review and meta-analysis. Clinical journal of the American Society of Nephrology. 2011 Oct 1;6(10):2364-73.

Tiwari-Heckler S, Gan-Schreier H, Stremmel W, Chamulitrat W, Pathil A. Circulating phospholipid patterns in NAFLD patients associated with a combination of metabolic risk factors. Nutrients. 2018 May 21;10(5):649.

Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. British journal of nutrition. 2004 Sep;92(3):347-55.

Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of clinical Biochemistry. 1969 Jan;6(1):24-7.

Tripathi BK, Srivastava AK. Diabetes mellitus: complications and therapeutics. Med Sci Monit. 2006 Jul 1;12(7):130-47.

Tsai MK, Wang HM, Shiang JC, Chen I, Wang CC, Shiao YF, Liu WS, Lin TJ, Chen TM, Chen YH. Sequence variants of ADIPOQ and association with type 2 diabetes mellitus in Taiwan Chinese Han population. The scientific world journal. 2014 Jan 1;2014.

Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. New England Journal of Medicine. 2001 May 3;344(18):1343-50.

Turer AT, Scherer PE. Adiponectin: mechanistic insights and clinical implications. Diabetologia. 2012 Sep;55(9):2319-26.

Unger RH. Minireview: weapons of lean body mass destruction: the role of ectopic lipids in the metabolic syndrome. Endocrinology. 2003 Dec 1;144(12):5159-65.

Van Dam RM, Willett WC, Rimm EB, Stampfer MJ, Hu FB. Dietary fat and meat intake in relation to risk of type 2 diabetes in men. Diabetes care. 2002 Mar 1;25(3):417-24.

Van de Voorde J, Pauwels B, Boydens C, Decaluwé K. Adipocytokines in relation to cardiovascular disease. Metabolism. 2013 Nov 1;62(11):1513-21.

Van Gaal LF, Mertens IL, De Block CE (2006) Mechanisms linking obesity with cardiovascular disease. Nature 444(7121): 875–880.

Vandewalle CL, Coeckelberghs MI, De Leeuw IH, Caju MV, Schuit FC, Pipeleers DG, Gorus FK. Epidemiology, Clinical Aspects, and Biology of IDDM Patients Under Age 40 Years: Comparison of data from Antwerp with complete ascertainment with data from Belgium with 40% ascertainment. The Belgian Diabetes Registry. Diabetes care. 1997 Oct 1;20(10):1556-61.

Verma S, Li SH, Wang CH, Fedak PW, Li RK, Weisel RD, Mickle DA. Resistin promotes endothelial cell activation: further evidence of adipokine-endothelial interaction. Circulation. 2003 Aug 12;108(6):736-40.

Vionnet N, Dupont S, Gallina S, Francke S, Dotte S, De Matos F, Durand E, Leprêtre F, Lecoeur C, Gallina P, Zekiri L. Genomewide search for type 2 diabetes–susceptibility genes in French Whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2–diabetes locus on chromosome 1q21–q24. The American Journal of

Wang, H., Zhang, H., Jia, Y., Zhang, Z., Craig, R., Wang, X. and Elbein, S.C., 2004. Adiponectin receptor 1 gene (ADIPOR1) as a candidate for type 2 diabetes and insulin resistance. Diabetes, 53(8), pp.2132-2136.

Wannamethee SG, Shaper AG, Walker M. Overweight and obesity and weight change in middle aged men: impact on cardiovascular disease and diabetes. Journal of Epidemiology & Community Health. 2005 Feb 1;59(2):134-9.

Warren B, Pankow JS, Matsushita K, Punjabi NM, Daya NR, Grams M, Woodward M, Selvin E. Comparative prognostic performance of definitions of prediabetes: a prospective cohort analysis of the Atherosclerosis Risk in Communities (ARIC) study. The lancet Diabetes & endocrinology. 2017 Jan 1;5(1):34-42.

Weickert MO, Pfeiffer AF. Impact of dietary fiber consumption on insulin resistance and the prevention of type 2 diabetes. The Journal of nutrition. 2018 Jan 1;148(1):7-12.

Weisman A, Fazli GS, Johns A, Booth GL. Evolving trends in the epidemiology, risk factors, and prevention of type 2 diabetes: a review. Canadian Journal of Cardiology. 2018 May 1;34(5):552-64.

Wen CP, Cheng TY, Tsai SP, Hsu HL, Wang SL. Increased mortality risks of pre-diabetes (impaired fasting glucose) in Taiwan. Diabetes Care. 2005 Nov 1;28(11):2756-61.

Wen L, Ge H, Qiao J, Zhang L, Chen X, Kilby MD, Zhou Y, Gan J, Saffery R, Yan J, Tong C. Maternal dietary patterns and risk of gestational diabetes mellitus in twin pregnancies: a longitudinal twin pregnancies birth cohort study. Nutrition journal. 2020 Dec;19(1):1-2.

WHO, International Diabetes Foundation. Defi nition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation. Geneva: World Health Organization, 2006.

Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes care. 2004 May 1;27(5):1047-53.

Willi C, Bodenmann P, Ghali WA, Faris PD, Cornuz J. Active smoking and the risk of type 2 diabetes: a systematic review and meta-analysis. Jama. 2007 Dec 12;298(22):2654-64.

Willmann C, Brockmann K, Wagner R, Kullmann S, Preissl H, Schnauder G, Maetzler W, Gasser T, Berg D, Eschweiler GW, Metzger F. Insulin sensitivity predicts cognitive decline in individuals with prediabetes. BMJ Open Diabetes Research and Care. 2020 Nov 1;8(2):e001741.

Wilmot E.G., Edwardson C.L., Achana F.A., Davies M.J., Gorely T., Gray L.J., Khunti K., Yates T., Biddle S.J.H., Davies M. Sedentary time in adults and the association with diabetes, cardiovascular disease and death: Systematic review and meta-analysis. Diabetologia. 2012;55:2895–2905. doi: 10.1007/s00125-012-2677-z

Wolf, G.. Insulin Resistance in Type 2 Diabetes Current Molecular Medicine, 2005, Vol. 5, No. 3 339., (2004) Nutr. Rev., 62, 389-394.

Wu X, Motoshima H, Mahadev K, Stalker TJ, Scalia R, Goldstein BJ. Involvement of AMP-activated protein kinase in glucose uptake stimulated by the globular domain of adiponectin in primary rat adipocytes. Diabetes. 2003 Jun 1;52(6):1355-63.

X. Cai, L. Xia, Y. Pan, D. He, H. Zhu, T. Wei, Y. He, Differential role of insulin resistance and β-cell function in the development of prediabetes and diabetes in middle-aged and elderly Chinese population. Diabetol. metab. syndr. 11(1) (2019) 1-8.

Xu JY, Sham PC, Xu A, Tso AW, Wat NM, Cheng KY, Fong CH, Janus ED, Lam KS. Resistin gene polymorphisms and progression of glycaemia in southern Chinese: a 5-year prospective study. Clinical endocrinology. 2007 Feb;66(2):211-7.

Xu M, Li XY, Wang JG, Wang XJ, Huang Y, Cheng Q, Huang HE, Li R, Xiang J, Tan JR, Dai M. Retinol-binding protein 4 is associated with impaired glucose regulation and microalbuminuria in a Chinese population. Diabetologia. 2009 Aug;52(8):1511-9.

Xu SS, Hua J, Huang YQ, Shu L. Association between dietary patterns and chronic kidney disease in a middle-aged Chinese population. Public health nutrition. 2020 Apr;23(6):1058-66.

Yadav A, Jyoti P, Jain SK, Bhattacharjee J. Correlation of Adiponectin and Leptin with Insulin Resistance: A Pilot Study in Healthy North Indian Population. Indian J Clin Biochem. 2011;26(2):193–6. doi:10.1007/s12291-011-0119-1.

Yaffe K, Blackwell T, Whitmer RA, Krueger K, Barrett-Connor E. Glycosylated hemoglobin level and development of mild cognitive impairment or dementia in older women. The journal of nutrition, health & aging. 2006 Jul 1;10(4):293.

Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature. 2003 Jun;423(6941):762-9.

Yang W, Lu J, Weng J, Jia W, Ji L, Xiao J, Shan Z, Liu J, Tian H, Ji Q, Zhu D. Prevalence of diabetes among men and women in China. New England journal of medicine. 2010 Mar 25;362(12):1090-101.

Yeboah J, Bertoni AG, Herrington DM, Post WS, Burke GL. Impaired fasting glucose and the risk of incident diabetes mellitus and cardiovascular events in an adult population: MESA (Multi-Ethnic Study of Atherosclerosis). Journal of the American College of Cardiology. 2011 Jul 5;58(2):140-6.

Ying J, Shi NS, Pan RW, Li PZ, Zhang HQ. Association between Gln223Arg and type 2 diabetes in Zhejing. Chin Gerontol. 2009;29(7):858-60.

Yoshikawa H, Hellström-Lindahl E, Grill V. Evidence for functional nicotinic receptors on pancreatic β cells. Metabolism. 2005 Feb 1;54(2):247-54.

Youn BS, Yu KY, Park HJ, Lee NS, Min SS, Youn MY, Cho YM, Park YJ, Kim SY, Lee HK, Park KS. Plasma resistin concentrations measured by enzyme-linked immunosorbent assay using a newly developed monoclonal antibody are elevated in individuals with type 2 diabetes mellitus. The Journal of Clinical Endocrinology & Metabolism. 2004 Jan 1;89(1):150-6.

Yudkin JS, Montori VM. The epidemic of pre-diabetes: the medicine and the politics. Bmj. 2014 Jul 15;349.

Yun JE, Kimm H, Choi YJ, Jee SH, Huh KB. Smoking is associated with abdominal obesity, not overall obesity, in men with type 2 diabetes. Journal of Preventive Medicine and Public Health. 2012 Sep;45(5):316.

Zhang X, Gregg EW, Williamson DF, Barker LE, Thomas W, Bullard KM, Imperatore G, Williams DE, Albright AL. A1C level and future risk of diabetes: a systematic review. Diabetes care. 2010 Jul 1;33(7):1665-73.

Zhou X, Chen R, Zhong C, Wu J, Li X, Li Q, Cui W, Yi N, Xiao M, Yin H, Xiong G. Maternal dietary pattern characterised by high protein and low carbohydrate intake in pregnancy is associated with a higher risk of gestational diabetes mellitus in Chinese women: a prospective cohort study. British Journal of Nutrition. 2018 Nov;120(9):1045-55.

Zhuo X, Zhang P, Barker L, Albright A, Thompson TJ, Gregg E. The lifetime cost of diabetes and its implications for diabetes prevention. Diabetes care. 2014 Sep 1;37(9):2557-64.

Ziemke F, Mantzoros CS. Adiponectin in insulin resistance: lessons from translational research. The American journal of clinical nutrition. 2010 Jan 1;91(1):258S-61S.

Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature. 2001 Dec;414(6865):782-7.