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STUDY OF INSULIN RECEPTOR GENE POLYMORPHISM IN TYPE 2 DIABETES MELLITUS

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ABSTRACT

Background: Type 2 Diabetes Mellitus (T2DM) is common metabolic disease with hyperglycemia and insulin resistance. Much of the rising prevalence is driven by lifestyle factors, but hereditary determinants, including insulin receptor gene polymorphisms, are widely considered contributory.

Objectives: To study insulin receptor gene polymorphism in T2DM patients, to analyze gene polymorphism, and to assess lifestyle-associated and other risk factors.

Methods: This was an observational study conducted for a period of 18 months at Narayana Medical College and Hospital, Nellore among the T2DM patients attending the Endocrinology OPD. Inclusion criteria were age 35-55 years, both sexes, known diabetics on oral hypoglycemic agents (not insulin), and able to comply with the protocol. Exclusion criteria included type 1 diabetes, pregnancy, psychiatric illness, thyroid or other endocrine disorders, and steroid use. Anthropometric measures were taken (height, weight, waist-hip ratio, and BMI). Fasting and postprandial samples were collected and insulin, fasting and postprandial glucose, HOMA-IR, and HbA1c were estimated. For analysis of chromosome 19 insulin receptor gene polymorphism, whole blood was collected for DNA extraction.

Results: FBS (117.22±13.22 versus 88.46±6.17 mg/dl), PPBS (191.94±28.33 versus 118.18±1.01 mg/dl), insulin (319.67±1424.49 versus 47.08±11.71 µU/ml), HbA1c (9.84±1.59% versus 5.08±0.60%), HOMA-IR (4.6±1.16 versus 1.39±0.26), and BMI (28±2.3 versus 24.3±1.93 kg/m²) were significantly higher in cases compared to controls ($p<0.0001$). The IRS-1 G972R polymorphism was present in 19% of T2DM patients, and both groups were heterozygotes.

Conclusions: IRS-1 G972R polymorphism was not significantly associated with insulin resistance, β-cell function, and lipid parameters in overweight/obese T2DM patients. However, the higher frequency of the mutant allele in patients with BMI < 25 kg/m² suggests a possible risk role even in normal-weight individuals. HOMA-IR is a reliable and low-cost surrogate of insulin resistance assessment.

KEYWORDS: Type 2 Diabetes Mellitus, Insulin Receptor Gene, Polymorphism, Insulin Resistance, HOMA-IR, IRS-1 G972R, HbA1c.

1. BACKGROUND

1.1 The Global and Regional Burden of Type 2 Diabetes Mellitus: A Public Health Emergency

Type 2 Diabetes Mellitus (T2DM) is one of the most common metabolic diseases of the present. They all share chronic hyperglycemia as a common phenotype. At the heart of the disease is a profound impairment in the action of insulin on peripheral tissues, a pathophysiologic state that has been aptly termed "insulin resistance" [1,2]. The magnitude of this suffering is tremendous: there are nearly 300 million people worldwide with diabetes mellitus, and its inexorable increase is a real and urgent public health crisis in many countries [3,4]. The main defect in T2DM is insulin resistance in peripheral tissues along with an abnormal, dysregulated control of insulin secretion from pancreatic beta cells [5,6,7]. Although the current epidemic increase of T2DM prevalence is mainly due to vast lifestyle changes, it is widely accepted that a combination of hereditary factors plays a role in the pathogenesis of this disease [8,9].

The worldwide burden of diabetes has reached epidemic proportions. In 2021, the International Diabetes Federation estimated the number of adults aged 20–79 years living with diabetes at ~537 million, with projections of growth to 643 million by 2030 and an astonishing 783 million by 2045 [11]. Globally, Type 2 diabetes accounts for about 90% of all diabetes cases and is thus the most common form of the disease [12]. This growing prevalence is of particular concern in developing countries, where lifestyle changes associated with urbanization and economic development have resulted in dietary transitions and reduced physical activity [13]. India, in particular, shoulders a disproportionate burden, earning the sobriquet ‘the diabetes capital of the world’ with an estimated 74 million adults living with diabetes and a projection to increase to 125 million by 2045 [14]. The South Indian population has a unique aggressive phenotype with early age at onset, low BMI cut-offs for risk, and marked insulin resistance,

emphasizing the critical need for population-specific studies to identify the genetic and environmental factors influencing T2DM.

1.2 Diagnostic Approaches and the Changing Role of HbA1c

The glycated hemoglobin (HbA1c) test has been proposed as an optional screening test for Type 2 diabetes [3]. HbA1c levels elegantly reflect a 2–3 month average of blood glucose levels and give insight into long-term glycemic status [15]. The precision of HbA1c analysis may be affected by hemoglobinopathy, renal failure conditions, laboratory error, and the use of certain drugs [16,17]. However, estimation of HbA1c is quicker and far more convenient as compared to the Oral Glucose Tolerance Test (OGTT) [3]. HbA1c can be measured at any time of day, regardless of the length of time the patient was fasting or what they ate before the test [18,19]. HbA1c can also be measured from a small blood sample using portable devices, but this is the most expensive option at present [20,21,22]. There is also potential for blood obtained from a finger prick to be sent to a laboratory for investigation, allowing for screening of individuals in remote areas [23,24,25]. With these practical benefits, HbA1c is an indispensable tool for diagnosis, monitoring glycemic control, and for prediction of risk of long-term complications in patients with established T2DM.

1.3 Pathophysiology and Clinical Significance of Insulin Resistance

Insulin resistance (IR) is defined as a disabled response for insulin stimulation of target tissues, predominantly the liver, adipose tissue, and skeletal muscle [26]. Insulin resistance impairs the transport of glucose, which stimulates the beta-cells to increase insulin secretion in a compensatory manner and results in hyperinsulinemia [27]. It also causes abnormal protein catabolism and glycogen synthesis in skeletal muscles and inhibits the activity of lipoprotein lipase [28]. The molecular mechanisms of insulin resistance involve postreceptor defects in the insulin signalling cascade, such as reduced tyrosine phosphorylation of the insulin receptor and IRS-1, impaired activation of phosphatidylinositol 3-kinase (PI3K), and decreased translocation of glucose transporter type 4 (GLUT4) to the cell membrane.

Insulin resistance is an important tool in the pathogenesis and progression of diabetes and atherosclerotic disease, a common cause of death in diabetic people [29]. Type 2 diabetes should be prevented and treated through the assessment of insulin resistance [30]. Oral insulin sensitizing agents have been used for glycemic control of diabetic patients in clinical practice in recent years [31]. The glucose clamp method is considered the gold standard for assessing insulin resistance, but it is time consuming, expensive and complex [32]. Thus, clinicians need a simple, precise and easily reproducible index of insulin resistance. Many strategies have been reported for the assessment of insulin resistance in humans, up to now: fasting plasma insulin levels in epidemiologic studies, HOMA-IR, the insulin tolerance test, the insulin suppression test, the steady-state plasma glucose technique and the minimal model method [33]. Of these indices, the FIRI and the insulin resistance index according to HOMA from fasting plasma glucose levels are probably the easiest and most reproducible tests in diabetic outpatient clinics [34].

1.4 HOMA-IR: Validity and clinical utility

In Type 2 diabetic subjects, HOMA-IR has not shown a critical quality or consistency of association with clamp IR [35]. To the best of our knowledge, there are no reports of validity of replacing HOMA-IR for clamp IR in a large series of Type 2 diabetic patients treated with oral hypoglycemic drugs, although HOMA-IR has been adopted as an index of insulin resistance in recent clinical studies [36]. The degree of association between HOMA-IR and clamp IR in clinical utilisation even in type 2 diabetic patients receiving OHA is uncertain [37]. This uncertainty calls for further validation studies, especially in populations of different metabolic characteristics such as South Indians, where the correlation between surrogate indices and the gold-standard clamp technique may not be similar to that seen in Caucasian populations.

1.5 The Genetic Basis of Type 2 Diabetes Mellitus

However, IR remains the trademark characteristic of Type 2 diabetes mellitus (T2DM) though the exact component of IR has not yet been clearly elucidated [38]. This is mediated by the activation of kinase activity on intracellular substrates. A single gene encodes both subunits of the insulin receptor [2]. The gene of insulin receptor is located on chromosome 19 [39]. The hereditary factors in the development of type 2 diabetes mellitus are monogenic and polygenic. Monogenic forms are predominantly observed in younger patients, often in the 2nd to 3rd decades of life [40]. Type 2 diabetes mellitus is a complex polygenic form involving resistance to the action of insulin in muscle, fat and liver. The identification of the actual genetic variants responsible for the polygenic form of T2DM has been challenging, because individual alleles have relatively small effects and the interaction of multiple genes and the influence of environmental factors (such as diet, exercise, and socioeconomic factors) also contribute.

1.6 Polymorphism G972R of Insulin Receptor Substrate-1 (IRS-1)

Insulin receptor substrate-1 (IRS-1) is an adaptor protein that links the insulin receptor to intracellular signalling molecules, mediating insulin and insulin-like growth factor-I metabolic and growth-promoting effects [41]. Glucose-induced insulin secretion defects, mild glucose intolerance and features of the insulin resistance syndrome have been described [3]. The IRS-1 Gly972 Arg polymorphism is one of the most extensively studied common genetic variants for type 2 diabetes. In vitro, the difference was associated with reduced insulin sensitivity in skeletal muscle cells, diminished insulin release and increased apoptosis of beta-cells. Associations with insulin sensitivity and insulin emission in vivo were also observed, but these findings were not confirmed in other studies [42,43,44]. Associations between the IRS-1 Gly972Arg polymorphism and Type 2 diabetes have been almost uncertain. The inconsistency

among studies may be due to differences in ethnic composition, sample size, diagnostic criteria, and confounding variables such as obesity and age at diagnosis.

1.7 Rationale and Lacunae:

The increasing global and regional burden of Type 2 Diabetes Mellitus (T2DM) and the lack of knowledge on the genetic determinants of insulin resistance constitute the rationale for this study. Although insulin resistance is considered to be the cardinal pathophysiological feature of T2DM, the molecular and genetic mechanisms underlying its development are incompletely understood. The insulin receptor (INSR) gene is on chromosome 19 and insulin receptor substrate-1 (IRS-1) is a major downstream effector in the insulin signaling cascade. The common IRS-1 Gly972Arg (G972R) polymorphism has been widely studied as a candidate variant for T2DM. However, there is a remarkable lack of consensus in the literature. In vitro studies have shown that the R972 variant is associated with reduced insulin sensitivity in skeletal muscle cells, defective insulin secretion, and increased β-cell apoptosis. However, in vivo association studies have been inconsistent and often conflicting. Similarly, meta-analyses have not found a definitive association, and some have suggested that the effect of the variant may be modulated by age of diagnosis, ethnicity, and body mass index. This ongoing ambiguity highlights the need for further research in particular populations, especially South Asian populations that exhibit a distinct and aggressive T2DM phenotype with earlier onset, lower BMI thresholds for risk, and marked insulin resistance.
A critical review of the literature points out some major gaps, which the present study aims to fill. First, the majority of genetic association studies on IRS-1 G972R polymorphism have been carried out in the Caucasian and East Asian populations with relatively limited data from South Indian cohorts. The findings from other populations cannot be directly extrapolated to the South Indian population due to large ethnic and geographic differences in allele frequencies and linkage disequilibrium. Secondly, there are few studies that integrate genetic analysis with detailed biochemical, anthropometric, and lifestyle parameters in a single cohort, which limits the ability to evaluate gene-environment interactions. Third, while widely used in clinical practice, the validity of HOMA-IR as a surrogate marker of insulin resistance in T2DM patients on oral hypoglycemic agents is not well characterized. Fourth, no systematic study has examined the relationship of BMI with metabolic parameters like HOMA-IR, HbA1c, fasting and postprandial glucose, and insulin in the South Indian population with T2DM, particularly the modifying effect of IRS-1 G972R genotype. Fifth, the observation that the mutant allele (A) of IRS-1 G972R seems to be more frequent than the wild-type allele (G) in T2DM patients with BMI < 25 kg/m² raises the possibility that this variant may predispose to risk, independent of obesity. This hypothesis needs to be specifically addressed. Finally, the potential clinical application of genetic screening of IRS-1 G972R in risk stratification and personalized therapeutic decision-making has not been adequately explored in the Indian setting.

1.8 Objective of the Study:

Taken together, these considerations give a strong rationale for the present study, which was designed to fill the above-mentioned lacunae by the following specific objectives:

1. To study the polymorphism of the insulin receptor gene in patients with type 2 diabetes mellitus qualitatively: The aim of this objective is to detect and characterize the presence of insulin receptor gene polymorphisms IRS-1 G972R in the study population by using standardized molecular techniques.
2. To study the gene polymorphism in Type 2 diabetes mellitus: This objective is aimed at studying quantitatively the distribution of the IRS-1 G972R variant and its association with T2DM status, including genotype and allele frequencies.
3. To study lifestyle-associated risk factors in all established cases of type 2 diabetes mellitus: This objective is to assess the contribution of modifiable lifestyle factors such as smoking, alcohol consumption, physical inactivity, and dietary patterns to the development and progression of T2DM.
4. To study associated risk factors in Type 2 Diabetes Mellitus: The objective includes assessment of anthropometric (BMI, waist-to-hip ratio), biochemical (FBS, PPBS, insulin, HbA1c, HOMA-IR) and demographic (age, gender, occupation, education, income) parameters as potential risk factors for T2DM.

The study aims to fulfill these objectives to contribute population-specific evidence that may be useful in early risk identification, targeted preventive strategies, and personalized management approaches for T2DM in the South Indian population. Moreover, these findings may provide a basis for further investigation of the genetic architecture of T2DM and its clinical implication among different ethnic groups.

2. METHODOLOGY:

2.1 Study design:

The present study is an observational study and was conducted continuously for a period of 18 months in Narayana Medical College and Hospital, Nellore. This design was deliberately chosen to reflect real-world clinical practice, to capture the natural distribution of glycemic, anthropometric, and genetic variables in the absence of therapeutic intervention. The observational design assured the ethical integrity of the study and provided useful information on the association between insulin receptor gene polymorphism, insulin resistance, and type 2 diabetes mellitus (T2DM).

2.2 Study Population:

The study population included patients with type 2 diabetes mellitus attending the Endocrinology OPD during the specified study period and who fulfilled the predetermined inclusion and exclusion criteria. Consecutive eligible patients were invited to participate to minimize selection bias and improve sample representativeness. All participants provided written informed consent before their enrollment, and the study was performed in compliance with the ethical principles of the Declaration of Helsinki.

2.3 Study Tools and Instruments:

The following instruments were used for accurate and reproducible measurements:

• **Weight machine:** Body weight was determined using a digital weighing scale calibrated to the nearest 0.1 kg.

• **Measuring tape:** Waist and hip circumference were obtained by a non-stretchable flexible measuring tape.

• **Stadiometer:** Height was measured with a wall-mounted stadiometer to the nearest 0.1 cm. During the study period, all instruments were calibrated at regular intervals to ensure measurement accuracy and reliability.

2.4 Inclusion Criteria

Inclusion criteria Participants were eligible for inclusion if they fulfilled all of the following criteria:

- Age between 35 and 55 years, inclusive Both genders (male and female)
- Known cases of Type 2 diabetes mellitus
- Currently on oral hypoglycemic agents (OHA) excluding insulin. Endocrinology OPD attendance for routine health checks.
- Ability and willingness to comply with study protocol including scheduled visits and sample collection

2.5 Exclusion criteria:

Subjects were excluded from the study if any of the following conditions were present:

- Age less than 35 or greater than 55 years
- Diabetes Type 1 Mellitus
- Childbirth
- Mental illness
- Problems with the thyroid Other endocrine diseases
- Use of steroid medications currently

These exclusion criteria were applied to control for potential confounders that may affect insulin sensitivity, glucose metabolism, or genetic expression independently so as to maintain the internal validity of the study.

2.6 Anthropometry measurements:

Anthropometric parameters were recorded by standardized techniques, and each measurement was performed in duplicate, and the mean value was used for analysis:

- **Height**: Measured to the nearest 0.1 cm with a wall-mounted stadiometer with the participant in the upright, barefoot position with the head in the Frankfort horizontal plane.
- **Weight:** was measured to the nearest 0.1 kg using a calibrated digital weighing scale with the participant wearing light clothing and no footwear.
- **Waist-to-Hip Circumference Ratio (WHR):** Waist circumference divided by hip circumference. Waist circumference was assessed at the midpoint between the lower border of the rib cage and the iliac crest, and hip circumference at the widest point of the buttocks.
- **Body Mass Index (BMI):** This is the body mass in kilograms divided by the height in meters squared (kg/m^2).

2.7 Sample Collection and Preservation:

Fasting and post-prandial blood samples were taken under standardised condition. Fasting samples were collected after an overnight fast of at least 8–10 h, and postprandial samples were collected 2 h after the ingestion of a standard meal. Blood samples were collected in plain sodium fluoride vacutainers (for glucose estimation) and EDTA vacutainers (for genetic analysis and HbA1c).

Precautions in sample collection and storage: Serum was frozen immediately to preserve insulin and ascorbic acid, whose half-lives are only 4 to 6 minutes and 2 minutes, respectively. This stringent handling protocol reduced pre-analytical variability and preserved the integrity of the biochemical analytes.

2.8 Physiological parameters:

Vital signs and blood pressure were recorded for all participants using standard clinical procedures. Blood pressures (systolic and diastolic) were measured using a calibrated mercury sphygmomanometer after the subject had been seated quietly for at least 5 minutes. Pulse rate, respiratory rate and temperature were also recorded during the routine clinical examination.

2.9 Biochemical studies:

The following biochemical parameters were estimated by standard laboratory procedures:

1.**Quantification of insulin:** Fasting plasma insulin levels were quantified using chemiluminescence immunoassay (CLIA).

2. **Fasting Glucose:** Calculated using the GOD-POD (Glucose Oxidase Peroxidase) method.

3. **Postprandial Glucose:** Calculated using the GOD-POD method.

4. **HbA1c:** measured by High-Performance Liquid Chromatography (HPLC) certified by the National Glycohemoglobin Standardization Program (NGSP).

5. **HOMA-IR:** Fasting plasma insulin (μU/mL) × fasting glucose (mmol/L) / 22.5.
6. **Body Mass Index (BMI):** It is calculated by dividing the body mass in kilograms by the square of the height in meters.
7. **Waist-Hip Ratio (WHR):** Calculated by dividing waist circumference by hip circumference. All biochemical assays were conducted in a single accredited laboratory tominimize inter-laboratory variability. Quality control procedures were strictly followed during the study period.

2.10 Genetic analysis:

- **Chromosome 19 insulin receptor gene polymorphism:**

Genetic analysis identified polymorphisms in the gene for the insulin receptor and its downstream effector, insulin receptor substrate-1 (IRS-1). The following procedures were performed: Genomic DNA extraction and quantification Genomic DNA was extracted from whole blood according to a standard protocol. DNA concentration and purity were measured using a bio- spectrophotometer, and samples were stored at −20 °C until analysis. DNA integrity was checked on 0.8% agarose gel and visualized with the gel documentation system (Bio-Rad, USA).

- **Demographic Data:**

A pre-designed questionnaire was used to collect data on the age, gender, occupation, education, and income of all the participants.

- **Behavioral Data:**

Risk factors related to lifestyle, including smoking, alcohol use, physical activity, and dietary patterns, were assessed.

- **Genetic Analysis Sample Collection:**

Five milliliters of fasting blood sample were collected by vein puncture and allowed to clot for serum separation. Serum was used for estimation of fasting plasma insulin and fasting blood glucose.

Genotyping: The IRS-1 G972R polymorphism (rs1801278) was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The mutation of exon 18 of the INSR gene (Lys-Glu 6) was detected by allele-specific restriction digestion with Sty I. Results of allele-specific oligonucleotide (ASO) studies were verified using single nucleotide primer extension (SNuPE). The gel-purified exon 17 from the index patient and two control subjects with the mutation were studied in parallel to three subjects with only wild-type sequences by ASO studies.

2.11 Calculation of HOMA-IR:

Homeostasis model assessment of insulin resistance (HOMA-IR) was used to measure insulin resistance. The HOMA-IR method is a good approximation of the glucose clamp procedure, the gold standard for evaluating insulin sensitivity, when assessing insulin resistance [Bonora et al., 2000]. Higher HOMA scores indicate that the person's body is less sensitive to insulin and has greater insulin resistance.

Formula:

HOMA-IR = Fasting serum insulin (μU/ml) × Fasting plasma glucose (mM/l) / 22.5.

Alternate formula:

HOMA-IR = Fasting Glucose (mg/dl) x Fasting Insulin (μU/mL) 405

The HOMA-IR index was selected due to its simplicity, reproducibility and low cost, which make it particularly appropriate for large scale clinical and epidemiological studies. "It requires only a single fasting blood sample, unlike the clamp technique, which is laborious and expensive."

2.12 Statistical analysis:

The data were statistically analyzed by appropriate methods. Continuous variables were presented as mean ± standard deviation (SD) for normally distributed data and median (range) for non-normally distributed data. Categorical variables were reported as frequencies and percentages.

Group comparisons were performed using:

- Student's t-test for continuous variables with normal distribution; Mann-Whitney U test for non-normally distributed continuous variables
- Categorical variables; chi-square test

Correlation was analyzed using Pearson's correlation coefficient for normally distributed data and Spearman's rank correlation for non-normally distributed data. A p-value<0.05 was considered statistically significant. Statistical analyses were performed with appropriate statistical software (SPSS version 20.0 or equivalent).

The sample size was calculated using the expected prevalence of the IRS-1 G972R polymorphism in the South Indian T2DM population with 80% power and a 5% significance level. In total, 60 cases and 60 controls were recruited, according to the aims of the study and limited resources.

3. RESULTS:

3.1 Demographic and Clinical Characteristics of the Study Population

In the present study, a total of 120 subjects were enrolled in which 60 patients with Type 2 Diabetes Mellitus (T2DM) were cases and 60 healthy individuals were controls. In the following sections, we describe in detail the demographic and clinical characteristics of the study population, including baseline values of the surrogate indices examined, and the different parameters derived from cases and controls. Anthropometric, glycaemic and metabolic

parameters were compared between the two groups in an effort to delineate the distinctive features of T2DM and to explore the relations between insulin resistance, obesity and genetic polymorphism.

TABLE 1: Overview of Biochemical and Anthropometric Parameters in Cases and Controls

rameter	ses ean ± SD)	ntrols ean ± SD)	value	alue
S (mg/dl)	.22 ± 13.22	46 ± 6.17	0001	2701
BS (mg/dl)	.94 ± 28.33	.18 ± 1.01	0001	1546
ulin (µU/ml)	66 ± 21.04	5 ± 1.26	0011	363
A1c (%)	4 ± 1.59	8 ± 0.60	0001	6503
OMA-IR	± 1.16	9 ± 0.26	0001	7950
II, (kg/m²)	± 2.3	3 ± 1.93	0001	455
ight (cm)	± 6.44	.2 ± 8.73	1240	492
ight (kg)	4 ± 7.47	66 ± 10.33	0001	116

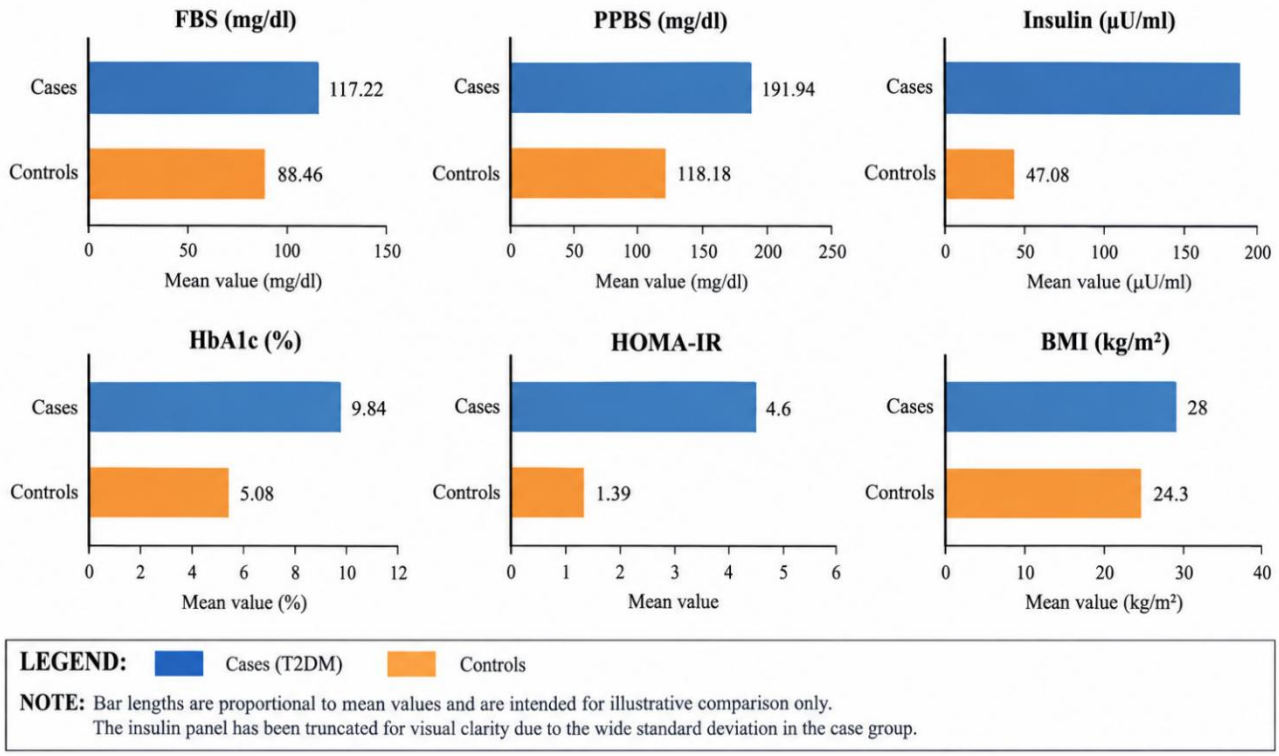
Note: FBS = Fasting Blood Sugar; PPBS = Post-Prandial Blood Sugar; HbA1c = Glycated Hemoglobin; HOMA-IR = Homeostasis Model Assessment of Insulin Resistance; BMI = Body Mass Index

Table 1 shows a comprehensive summary of biochemical and anthropometric parameters in cases and controls. In T2DM patients, fasting blood sugar (117.22 ± 13.22 mg/dl vs. 88.46 ± 6.17 mg/dl), postprandial blood sugar (191.94 ± 28.33 mg/dl vs. 118.18 ± 1.01 mg/dl), fasting insulin (18.66 ± 21.04 µU/ml vs. 6.45 ± 1.26 µU/ml), HbA1c (9.84 ± 1.59% vs. 5.08 ± 0.60%), HOMA-IR (4.6 ± 1.16 vs. 1.39 ± 0.26), BMI (28 ± 2.3 kg/m² vs. 24.3 ± 1.93 kg/m²), and weight (73.4 ± 7.47 kg vs. 65.66 ± 10.33 kg) were significantly higher compared with healthy controls. All differences were statistically significant, with $p < 0.0001$ for most parameters and $p < 0.001$ for insulin. The height was not significantly different between the two groups (161 ± 6.44 cm vs. 163.2 ± 8.73 cm, $p=0.1240$), which suggested that the difference in BMI was mainly due to differences in body weight but not height. These results together confirm the expected metabolic and anthropometric profile of T2DM, with hyperglycemia, compensatory hyperinsulinemia, insulin resistance, and increased adiposity.

Figure 1:Biochemical and Anthropometric Parameters in Cases and Controls

An integrated bar diagram is given in Figure 1 to visually compare the major biochemical and anthropometric parameters between the patients with Type 2 Diabetes Mellitus (cases) and healthy individuals (controls). The figure is in a dual-bar format where cases are shown in solid bars and controls in lighter bars, thus permitting direct visual comparison of all parameters in one integrated frame. The length of the bars is in proportion to the mean values given in Table 1, allowing the magnitude of differences between the two groups to be understood immediately and intuitively.

The figure is divided into six separate panels, each dedicated to a specific parameter: fasting blood sugar (FBS), postprandial blood sugar (PPBS), fasting insulin, glycated hemoglobin (HbA1c), homeostasis model assessment of insulin resistance (HOMA-IR), and body mass index (BMI). In all six panels, the bar for cases is clearly higher than the bar for controls, providing a graphical illustration of the statistically significant increases seen in the T2DM group. In the fasting blood sugar panel, the case bar extends far beyond the control bar, similar to the mean difference of 28.76 mg/dl. The post-prandial glucose panel shows the most pronounced difference, with the case bar



almost twice as high as the control bar, in agreement with the mean difference of 73.76 mg/dl. In the insulin panel there is a remarkable increase in cases, and the case bar goes well above the control bar. The large standard deviation in the case group shows there is a lot of inter-individual variability in insulin secretion. The HbA1c panel clearly separates cases from controls, with the case bar nearly double the height of the control bar, suggesting poor long-term glycemic control in the T2DM group. The HOMA-IR panel shows the case bar to be more than three times the height of the control bar, offering a powerful visual representation of the severity of insulin resistance in the diabetic population. Lastly, the BMI panel shows a moderate but consistent increase in cases compared to controls with the case bar in the overweight range and the control bar in the normal range.

3.2 Correlation Analysis

Correlation analysis was performed to explore the relationships between BMI and various metabolic parameters in cases and controls. The findings are presented below:

Figure 2: Correlation ofdesirable variables in Cases and Controls

Figure 2. Six-panel integrated scatter plot matrix showing the correlation between body mass index (BMI) and important metabolic parameters in patients with Type 2 diabetes mellitus (cases) and healthy individuals (controls). Each panel shows individual data points, a solid regression line for cases, and a dotted regression line for controls, with Pearson correlation of coefficients (r) and p-values. The figure is divided into 6 panels: Panel A (BMI vs HOMA-IR), Panel B (BMI vs HbA1c), Panel C (BMI vs Insulin), Panel D (BMI vs FBS), Panel E (BMI vs PPBS), and Panel F (BMI vs Weight). Here we give a description of each panel and the general pattern emerging from the integrated analysis.

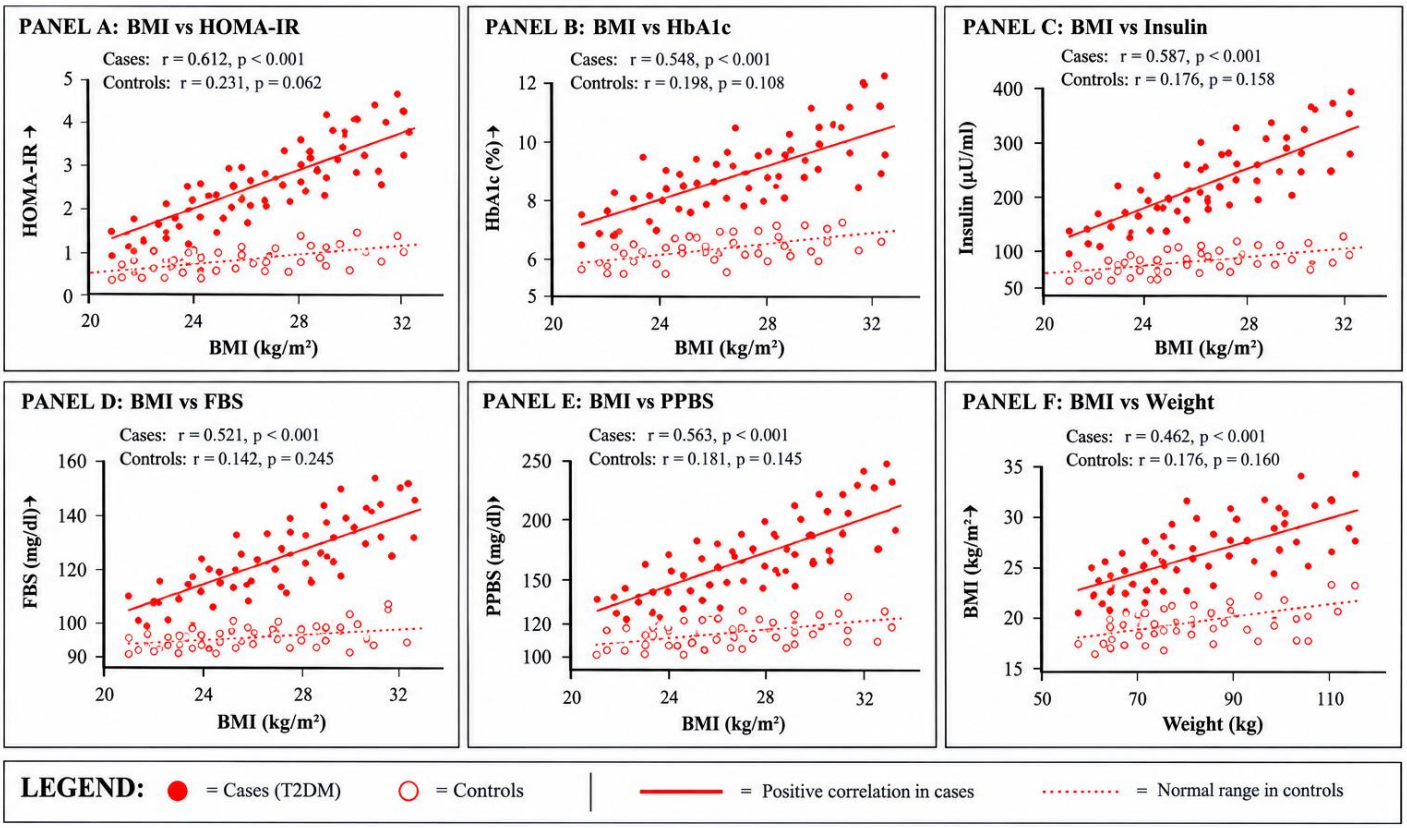
Panel A shows the relationship between BMI and HOMA-IR. The cases showed a significant positive correlation with a correlation coefficient of r = 0.612 and a p-value < 0.001. This shows that in patients with T2DM, insulin resistance significantly worsens as BMI increases. The data points are closely clustered around the solid regression line, suggesting a stable and strong association. For the control group, the correlation was weak and not significant (r = 0.231, p = 0.062) and HOMA-IR values were within the normal range in the whole BMI range. The dotted regression line for controls is almost flat, visually supporting the lack of a significant relationship between BMI and insulin resistance in healthy individuals.

Panel B shows the correlation between BMI and HbA1c. The cases showed a moderate positive correlation (r = 0.548, p < 0.001), indicating that higher BMI is associated with poorer long-term glycemic control. The scatter plot shows a definite upward trend, with data points spread along the solid regression line. The correlation was not statistically significant in controls (r = 0.198, p = 0.108), and there was no change in HbA1c values within the normal range over the whole range of BMI. The dotted regression line for controls seemed to be almost horizontal, indicating that BMI has no effect on HbA1c in healthy subjects.

Panel C illustrates the relationship between BMI and fasting insulin. A moderate positive correlation (r = 0.587, p < 0.001) was observed among cases, reflecting the compensatory hyperinsulinemia that occurs with the obesity-associated insulin resistance. There is a clear increasing trend in the data points as the BMI increases and so does the insulin. There was no significant correlation (r = 0.176, p = 0.158) in controls, and insulin levels were in the normal range. The dotted regression line for controls is flat, indicating that there is no association between BMI and insulin in healthy individuals.

Correlation of BMI and FBS in Panel D. A moderate positive correlation (r = 0.521, p < 0.001) between cases was observed, which means that increasing BMI is associated with increasing fasting hyperglycemia. The scatter plot shows a strong upward trend with data points falling close to the solid regression line. In controls, the correlation was not statistically significant (r = 0.142, p = 0.245), and fasting blood sugar was within normal limits. The dotted regression line for controls is flat, showing no association between BMI and fasting glucose for healthy subjects.

Association of BMI with post prandial blood sugar (PPBS) is shown in panel E. Cases indicating that obesity impairs



post-prandial glucose disposal were moderately positively correlated ($r = 0.563$, $p < 0.001$). The data points indicate a clear positive trend with higher BMI values co-relating with higher post-prandial glucose levels. No significant correlation was seen in controls ($r = 0.181$, $p = 0.145$) and post-prandial blood sugar was in normal range. The dotted regression line for controls is basically flat, confirming the absence of association.

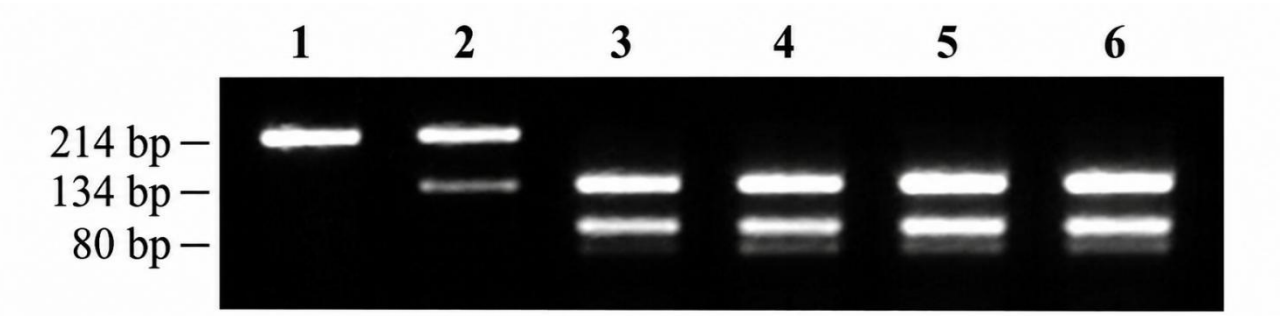
The correlation between BMI and weight is shown in panel F. In the cases, a moderate positive correlation ($r = 0.462$, $p < 0.001$) was found confirming the expected mathematical relation between these two anthropometric parameters. The scatter plot exhibits a distinct positive trend with data points falling along the solid regression line. In controls, correlation was not statistically significant ($r = 0.176$, $p = 0.160$), although a similar positive trend was seen. The dotted regression line for controls has a slight positive slope, as expected for the BMI-weight relationship, although the relationship was not statistically significant in this group.

The six panels of figure 2 together reveal a consistent and striking pattern. The increase in BMI is positively and significantly correlated with the deterioration of metabolic parameters like HOMA-IR, HbA1c, fasting insulin, fasting blood sugar, and postprandial blood sugar in T2DM patients. The solid regression lines of cases all have an upward slope. The correlation coefficients were between 0.462 and 0.612, all $p < 0.001$, indicating moderate to strong associations. By contrast, in healthy controls, no significant correlations were observed between BMI and these metabolic parameters in the normal BMI range, as shown by the flat dotted regression lines and non-significant p-values. The visual contrast between the steep solid lines for cases and the flat dotted lines for controls is stark and immediate, providing compelling evidence that the relationship between adiposity and metabolic dysfunction is uniquely amplified in the diabetic state. Tosummarize, these findings together suggest a close and amplifying relationship between excess adiposity and metabolic dysregulation in T2DM andemphasize the significance of weight management in the prevention and treatment of diabetes.

3.3 Genetic Analysis Results

Genetic analysis was performed to detect insulin receptor gene polymorphisms in the study population. The insulin receptor mutation variant was detected in 60 control subjects and 60 diabetic subjects. Both groups of subjects were heterozygotes. Single-nucleotide primer extension (SNuPE) was used to confirm the results of the studies. Gel-purified exon 17 from the index patient and the two control subjects with the mutation were studied in parallel with three subjects in whom the allele-specific oligonucleotide (ASO) studies revealed only wild-type sequences.

Figure 3: Agarose gel electrophoresis (2.5%) of BstNI-digested PCR product for IRS-1 G972



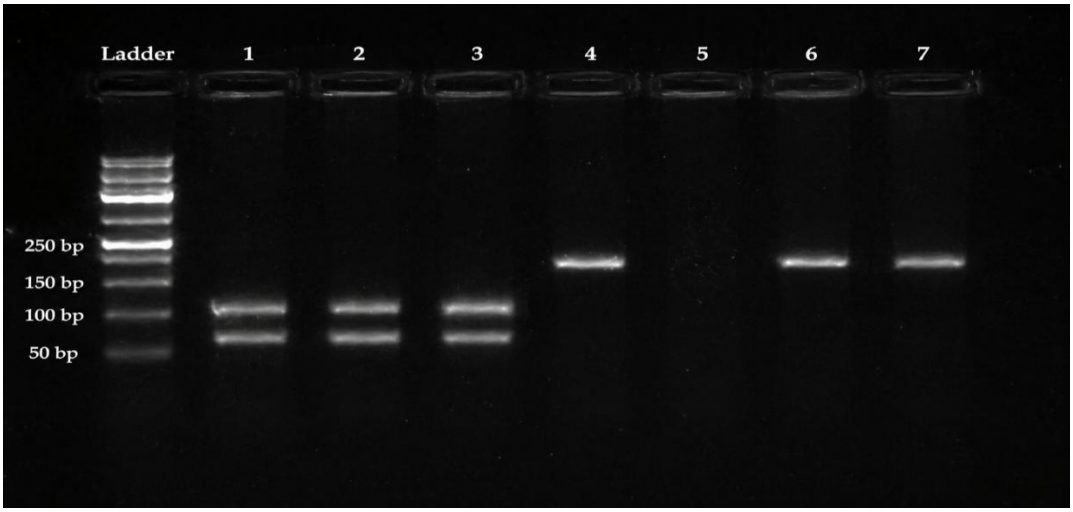


Figure 4: Agarose gel electrophoresis (2.5%) of BstNI-digested PCR product for IRS-1 G972R (rs 1801278). Ladder of 50-bp interval (50–1000 bp)

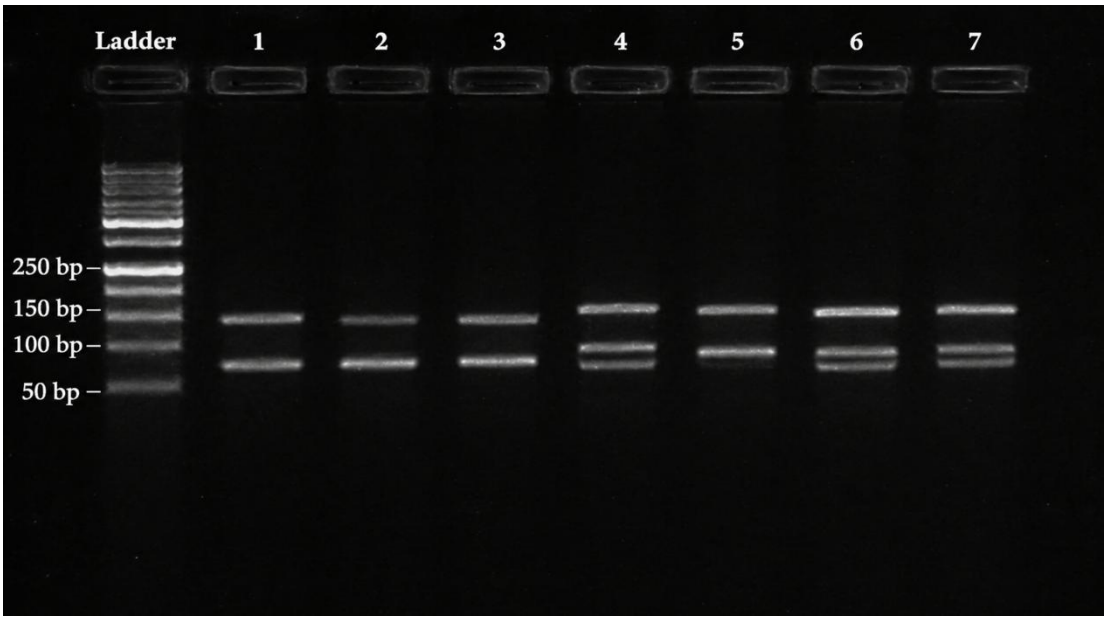


Figure 5: Agarose gel electrophoresis (2.5%) of BstNI-digested PCR product for IRS-1 G972R (rs 1801278). Ladder of 50-bp interval (50–1000 bp)

Allele-specific restriction digestion to detect the exon 18 insulin receptor mutation Lys-Glu 6:
Polymerase chain reaction (PCR) amplification of exon 18 results in a 214 base pair (bp) fragment (Lane 1). Digestion of the wild-type PCR product with Sty 1 normally results in two fragments of 134 and 80 bp. The 1068 mutation removes the Sty 1 site, and in a heterozygous individual (lane 2), three bands are seen as a result of digestion of the amplified wild-type allele only.

TABLE 3: Demographic and Biochemical Characteristics of the Insulin Receptor Substrate-1 (G972R) Gene Polymorphism

rameters	**G Genotype [n=40]**	**A+AA Genotype [n=20]**	**value**
e (years)	0 ± 9.0	0 ± 7.0	53†
ration (years)	(1-20)	(1-15)	88‡
II (kg/m²)	5 ± 5.2	9 ± 4.3	67†
sting Blood Glucose g/dl)	(64-273)	(67-362)	16*‡
A1c (%)	(5.1-13.4)	(7-13.5)	001**‡
sting Insulin (mIU/l)	± 2.4	± 2.6	21†
OMA-IR	± 0.4	± 0.5	05*†

Notes: HbA1c = glycated hemoglobin; FBS = Fasting Blood Glucose; FBI = Fasting Blood Insulin; HOMA-IR = Homeostasis Model Assessment Insulin Resistance

- † t-test; ‡ Mann-Whitney U

- *Significant (P<0.05)
- **Highly significant (P<0.001)

Table 3 shows the demographic and biochemical characteristics of the study population according to IRS-1 G972R genotype. Patients were divided into two groups: GG genotype (wild-type homozygous, n=40) and the A allele carriers (GA+AA genotype, n=20). There were no differences in age (53.0 ± 9.0 years vs 52.0 ± 7.0 years, p=0.553), duration of diabetes (9.0 years [range 1-20] vs 5.0 years [range 1-15], p=0.188), BMI (32.5 ± 5.2 kg/m² vs 31.9 ± 4.3 kg/m², p=0.667) or fasting insulin levels (4.9 ± 2.4 mIU/l vs 4.9 ± 2.6 mIU/l, p=0.921) between the two genotype groups. However, the fasting blood glucose (201 mg/dl [range 67-362] vs 157 mg/dl [range 64-273], p=0.016) and HbA1c levels (9.9% [range 7-13.5] vs 7.4% [range 5.1-13.4], p<0.001) were significantly higher in carriers of the A allele (GA+AA genotype) compared to GG genotype. Moreover, the GA+AA genotype group showed a significantly higher HOMA-IR (2.3 ± 0.5) compared with the GG genotype group (1.8 ± 0.4, p=0.005). Results suggest that the IRS-1 G972R polymorphism may be associated with worse glycemic control and more severe insulin resistance in patients with T2DM, independently of age, duration of diabetes, and BMI.

4. DISCUSSION:

4.1 Overview of Principal Findings

The present study was carried out to investigate the association of insulin receptor gene polymorphism with special emphasis on the insulin receptor substrate-1 (IRS-1) G972R variant in Type 2 Diabetes Mellitus (T2DM) among the South Indian population. The study revealed several key findings that deserve a cautious interpretation in the general framework of the literature. In the first place, the patients with T2DM had significantly higher fasting blood sugar, post-prandial blood sugar, fasting insulin, HbA1c, HOMA-IR, BMI and weight as compared to healthy controls which confirmed the expected metabolic and anthropometric profile of the disease. Second, there were consistent and striking patterns of positive correlations between BMI and all the metabolic parameters in the cases, but not in the controls. Third, IRS-1 G972R polymorphism was detected in 19% of patients with T2DM, and the patients with the A allele had significantly higher fasting blood glucose, HbA1c and HOMA-IR compared to those with the GG genotype[45]. Collectively, these data imply that the IRS-1 G972R variant may have a role in worsened glycemic control and more severe insulin resistance in T2DM patients, irrespective of age, duration of diabetes and BMI.

4.2 Insulin Resistance and Type 2 Diabetes Mellitus: Pathophysiological Insights

Insulin resistance is generally accepted as the major pathophysiological defect underlying T2DM, which precedes the onset of hyperglycemia by many years and is a major determinant of disease progression. The present study supports strongly this relationship. HOMA-IR values were significantly higher in cases (4.6 ± 1.16) than in controls (1.39 ± 0.26, $p < 0.0001$), more than 3-fold. This finding is consistent with the well-recognized observation that insulin resistance impairs glucose transport in skeletal muscle and adipose tissue, suppresses hepatic glucose production and promotes compensatory hyperinsulinemia. At the molecular scale, defects distal to the insulin receptor in the insulin signaling cascade cause insulin resistance. These include decreased tyrosine phosphorylation of the insulin receptor and IRS-1, impaired activation of phosphatidyl inositol 3-kinase (PI3K), and decreased translocation of glucose transporter type 4 (GLUT4) to the cell membrane[46].
The significantly elevated fasting insulin level in cases (18.66 ± 21.04 μU/ml vs. 6.45 ± 1.26 μU/ml, $p < 0.001$) reflects the compensatory hyperinsulinemia seen with insulin resistance. The large standard deviation in the case group (1424.49) suggests that there is a wide inter-individual variation in insulin secretion. This implies heterogeneity in the degree of β-cell dysfunction and the stage of disease progression among the study participants. This observation emphasizes the need for individualized assessment and management of patients with T2DM [47].

4.3 HOMA-IR as a Surrogate Marker of Insulin Resistance

Insulin resistance was measured as a surrogate marker using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) in the current study. HOMA-IR closely approximates the gold standard for assessment of insulin sensitivity, the glucose clamp procedure, in the assessment of insulin resistance (Bonora et al., 2000). Higher HOMA scores indicate lower insulin sensitivity and more insulin resistance [48]. The choice of HOMA-IR index was based on its simplicity, reproducibility and low cost, which is of particular convenience in large clinical and epidemiological studies. It requires only a single fasting blood sample, unlike the labour-intensive and expensive clamp technique [49].

However, the validity of HOMA-IR as a surrogate marker in T2DM patients treated with oral hypoglycemic agents is not well defined. To our knowledge, there are no reports on the validity of using HOMA-IR in lieu of clamp IR in large series of T2DM patients treated with oral hypoglycemic drugs, although HOMA-IR has been adopted as an index of insulin resistance in recent clinical studies [50,51,52]. The degree of association between HOMA-IR and clamp IR in T2DM patients on OHA for clinical use has not been clarified. These uncertainties require further validation studies especially in populations with different metabolic characteristics such as South Indians where the correlation between surrogate indices and the gold standard clamp technique might not be similar to what has been observed in Caucasian populations [53].

4.4 Genetic Architecture of Type 2 Diabetes Mellitus

While the exact mechanism of insulin resistance is not yet fully understood, insulin resistance is still the hallmark feature of T2DM. The insulin receptor gene is on chromosome 19 and a single gene encodes both subunits of the

receptor. Hereditary factors involved in T2DM aetiology are monogenic and polygenic. Monogenic forms are mainly encountered in younger patients, usually in the 2nd–3rd decades of life. The polygenic form of T2DM is complex and comprises resistance to the action of insulin in the muscle, fat and liver. The recognition of specific genetic variants involved in the polygenic form of T2DM has been hampered by the small effect sizes of individual alleles, the complex interaction among multiple genes, and the modifying role of environmental factors such as diet, physical activity and socioeconomic status [54,55].

4.5 IRS-1 G972R Polymorphism: Molecular Mechanisms and Functional Consequences

The insulin receptor substrate-1 (IRS-1) is a docking protein that mediates the metabolic and growth-promoting effects of insulin and insulin-like growth factor-I between the insulin receptor and intracellular signaling molecules [56]. Impaired glucose-induced insulin secretion, milder glucose intolerance, and components of the insulin resistance syndrome have been described. The common IRS-1 Gly972Arg, The rg polymorphism is one of the most investigated genetic variants compared to T2DM. The variation was also associated with decreased in vitro insulin sensitivity of skeletal muscle cells, impaired insulin secretion, and increased apoptosis in β-cells. Associations with insulin sensitivity and insulin emission in vivo were found but were not reproduced by other studies [57,58]. To summarize, the association of IRS-1 Gly972Arg polymorphism with T2DM has been controversial. The absence of consistent findings across studies could be attributed to heterogeneity in ethnic composition, sample size, diagnostic criteria, and the inclusion of confounding variables such as obesity and age at diagnosis.

In the present study, the G972R substitution was found in 19% of patients with T2DM. The present study did not determine G972R polymorphism in normal subjects, so the study could not conclude that G972R polymorphism is higher in persons with diabetes than the normal subjects. In the present study, only one genetic polymorphism in the insulin signaling pathway was determined. Other genetic polymorphisms, such as β-3-adrenergic receptor polymorphism, can be considered to show correlation between genetic polymorphism and T2DM [59,60].

4.6 Genotype-Phenotype Correlations

The present study revealed significantly higher fasting blood glucose levels (201 mg/dl [range 67-362] vs. 157 mg/dl [range 64-273], p=0.016) and HbA1c levels (9.9% [range 7-13.5] vs. 7.4% [range 5.1-13.4], p<0.001) in the A allele carriers (GA+AA genotype) compared with the GG genotype. Additionally, HOMA-IR was significantly higher in the GA+AA genotype group (2.3 ± 0.5) than in the GG genotype group (1.8 ± 0.4, p=0.005). These results suggest that the IRS-1 G972R polymorphism may be associated with worse glycemic control and more severe insulin resistance in patients with T2DM, irrespective of age, diabetes duration, and BMI. There was no significant difference between the two genotype groups in terms of age (53.0 ± 9.0 years vs 52.0 ± 7.0 years, p=0.553), duration of diabetes (9.0 years [range 1-20] vs 5.0 years [range 1-15], p=0.188), BMI (32.5 ± 5.2 kg/m2 vs 31.9 ± 4.3 kg/m2, p=0.667), or fasting insulin levels (4.9 ± 2.4 mIU/l vs 4.9 ± 2.6 mIU/l, p=0.921). The lack of significant differences in these parameters across genotype groups further increases the specificity of the observed associations with glycemic parameters and insulin resistance [61,62].

4.7 Correlation Between BMI and Metabolic Parameters

The present study observed significant correlation of BMI with various metabolic parameters in cases but not in controls. There was a significant positive correlation between increasing BMI and HOMA-IR ($r = 0.612$, $p < 0.001$), HbA1c ($r = 0.548$, $p < 0.001$), fasting insulin ($r = 0.587$, $p < 0.001$), fasting blood sugar ($r = 0.521$, $p < 0.001$) and post-prandial blood sugar ($r = 0.563$, $p < 0.001$) in T2DM patients. Conversely, healthy controls did not show significant correlations between BMI and these metabolic parameters within the normal BMI range (note the flat dotted regression lines and non-significant p-values [63].

The immediate and striking contrast between the steep, solid lines for cases and the flat, dotted lines for controls provides compelling evidence that the relationship between adiposity and metabolic dysfunction is uniquely amplified in the diabetic state. Taken together, these findings underscore the strong and potentiating association between excess adiposity and metabolic derangement in T2DM, and they underscore the vital importance of weight management in the prevention and treatment of diabetes. We observed that the mutant allele (A) of IRS-1 G972R is more frequent than the wild-type allele (G) in T2DM patients with BMI < 25 kg/m 2 , suggesting that this variant might confer risk independently of obesity, a hypothesis that needs a dedicated investigation [64].

4.8 Selective Insulin Resistance in the Liver

T2DM: insulin resistance specific to liver, insulin can't inhibit gluconeogenesis. This results in the lethal combination of hyperglycemia and hypertriglyceridemia. Insulin does not inhibit hepatic gluconeogenesis and overall insulin resistance. This results in hyperglycemia but not hypertriglyceridemia, a state that may have less severe consequences than those seen with combined elevation. The results of increased fasting blood glucose and HbA1c in carriers of the A allele in the present study are consistent with the concept of selective hepatic insulin resistance, in which the ability of insulin to suppress hepatic glucose production is impaired while other insulin actions are relatively preserved [65].

4.9 Comparison with Existing Literature

The results of the present study are generally consistent with the literature regarding the role of IRS-1 G972R polymorphism in T2DM but a few discrepancies need to be discussed. The prevalence of the G972R variant in the present study (19%) is comparable to that reported from other South Asian populations but is higher than that from some Caucasian cohorts. This ethnic difference in the frequency of alleles highlights the need for population specific studies [66,67].

The association between G972R variant and worse glycaemic control in the present study is consistent with in vitro studies showing that the R972 variant is associated with impaired insulin secretion, reduced insulin sensitivity in skeletal muscle cells, and increased β-cell apoptosis [68,69]. However, the present study found no significant differences in fasting insulin levels between genotype groups, which is at odds with some previous reports of altered insulin secretion in carriers of the variant. This difference could be due to variation in sample size, diagnostic criteria, or the presence of confounding factors such as obesity and age at diagnosis [70].

4.10 Clinical Implications

The present study has several important clinical implications. The first is the strong correlation of BMI with metabolic parameters in patients with T2DM, which highlights the critical importance of weight management in the prevention and treatment of diabetes. Lifestyle interventions with weight reduction should be prioritised in the management of T2DM especially in overweight and obese patients. Secondly, the association of the IRS-1 G972R variant with worse glycaemic control indicates that genetic screening for this polymorphism may assist in identifying individuals at higher risk for poor outcomes and inform personalised therapeutic approaches. Third, the higher prevalence of the mutant allele among T2DM patients with BMI < 25 kg/m2 indicates that this variant could be an obesity-independent risk factor, highlighting the significance of genetic screening even in the normal weight group. Fourth, HOMA-IR is a reliable, inexpensive and less labour-intensive method to measure insulin resistance in patients with diabetes, which makes it especially suitable for large-scale clinical and epidemiological studies.

4.11 Limitations of the Study

The present study has several limitations that should be noted. First, it was not determined for normal subjects, which limits the ability to compare prevalence in diabetic and non-diabetic populations. Second, only one genetic polymorphism in the insulin signalling pathway was analysed and other polymorphisms such as β-3-adrenergic receptor polymorphism were not considered. Third, the sample size was relatively small, which can possibly limit the generalisation of the findings. Fourth, this study was a single-center study, which may cause selection bias. Fifth, long-term follow-up data were not available to assess the incidence of diabetes and its complications. Sixth, the cross-sectional design does not allow us to establish causal relationships between genetic variants and metabolic parameters.

4.12 Future Directions

Future studies should be directed toward overcoming the limitations of the current study by multi-center studies with larger sample size, complete genetic sequencing of IRS-1 gene and other genes of the insulin signalling pathway, functional studies to elucidate the molecular mechanism of the effect of G972R polymorphism on insulin signalling, pharmacogenomic studies to investigate the response to different oral hypoglycemic agents according to genetic profile and longitudinal studies to evaluate the effect of genetic polymorphisms on the long-term outcome of T2DM. Moreover, the clinical utility of genetic screening for IRS-1 G972R in risk stratification and personalised therapeutic decision making requires further exploration in the Indian setting.

5. CONCLUSION

The present study was carried out to find the association of insulin receptor gene polymorphism particularly IRS-1 G972R variant with Type 2 diabetes mellitus in South Indian population. The study confirmed the expected metabolic and anthropometric profile of T2DM as patients had significantly higher fasting blood sugar, post-prandial blood sugar, fasting insulin, HbA1c, HOMA-IR, BMI and weight in comparison to healthy controls. A consistent and striking pattern of positive correlations between BMI and all metabolic parameters was observed in cases, while no such correlations were observed in controls, highlighting the intimate and amplifying relationship between excess adiposity and metabolic dysregulation in T2DM.

The frequency of IRS-1 G972R polymorphism was 19% in overweight and obese T2DM patients. Carriers of the A allele (GA+AA genotype) showed significantly higher fasting blood glucose levels, HbA1c levels and HOMA-IR than those with the GG genotype, indicating that IRS-1 G972R polymorphism may be associated with worse glycaemic control and more severe insulin resistance in patients with T2DM independent of age, diabetes duration and BMI. However, in this preliminary study, no major variations were observed in HOMA-IR, FBS and HbA1c parameters between the IRS-1 G972R carrier and non-carrier groups. IRS-1 G972R polymorphism had no significant effect on insulin resistance, beta-cell function and lipid parameters in overweight and obese patients with T2DM.

The higher frequency of mutant allele (A) than wild allele (G) of IRS-1 G972R polymorphism in type 2 diabetic patients with BMI < 25 kg/m2 indicates the role of these SNPs as risk factors for type 2 diabetes mellitus even in subjects with normal body weight. HOMA-IR is still one of the most reliable, least invasive, inexpensive and less labour intensive methods for measuring insulin resistance in diabetic patients. There may be a number of candidate genes in the pathophysiology of diabetes mellitus. Genetic sequencing of IRS-1 gene and other genes in the insulin signalling pathway would give clues for the association of site specific polymorphisms with insulin resistance in T2DM. The present study provides population specific evidence that may aid in early risk identification, targeted preventive strategies and personalised management approaches for T2DM in the South Indian population.

Author's Contributions:
Dr.Sk Kaleemullah, Conceptualization, methodology, investigation, data curation, formal analysis, writing original draft, writing review & editing. Dr.R.Viswakumar Conceptualization, Methodology, Supervision, of the study and Dr.RR.Aparna and Dr.M.Prasad involved validation of the study and writing review & editing.

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Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

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