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ORIGINAL RESEARCH

Molecular Variants in *SIRT1* Gene Among Saudi Women Diagnosed with Gestational Diabetes Mellitus: A Case-Control Study

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Background: Gestational Diabetes Mellitus (GDM) is defined as impaired glucose intolerance resulting in hyperglycemia. SIRT1 deficiency and single nucleotide polymorphisms (SNPs) were also related to diabetes. No documented studies were carried out between GDM and *SIRT1* SNPs and it is also not confirmed whether *SIRT1* SNPs had a role in GDM women.

Objective: This study was designed to explore the molecular association carried out between GDM women and *SIRT1* gene SNPs present in the Saudi Arabia. This is a case-control study carried out in Capital city of Saudi Arabia.

Methods/Design: Serum samples were used for studying biochemical parameters, genotyping and Sanger sequencings for rs4746720 and rs10823112 SNPs. Statistical analysis was carried out between GDM and non-GDM women for anthropometric, biochemical and molecular data.

Results: A total of 120 GDM and 120 non-GDM women were recruited based on inclusion and exclusion criteria. Different forms of glucose values have confirmed the statistical association levels between GDM and non-GDM women ($p < 0.05$). Penalized logistic regression analysis showed the positive association with FBG, OGTT-2hr and HDLc levels ($p < 0.05$) in GDM women. The rs4746720 SNP was associated with GDM ($p = 0.01$). ANOVA analysis has confirmed the strong association with FBG ($p = 0.002$), PPBG ($p = 0.0001$) and HDLc ($p = 0.0003$) levels in rs4746720 SNP; while in rs10823112 SNP, both PPBG ($p = 0.0001$) and HDLc ($p = 0.01$) were associated. Linkage disequilibrium and GMDR analysis showed significant associations ($p < 0.05$).

Conclusion: This study confirms rs4746720 SNP and glucose levels played a role in this study.

Keywords: GDM, non-GDM women, SIRT1, rs4746720, rs10823112, SNPs

Introduction

The existence of elevated glucose tolerance levels for the first time during the second or third trimesters of pregnancy is characterized as gestational diabetes mellitus (GDM).[1] Alternatively, it can be defined as glucose or carbohydrate intolerances that are detected for the first time during the pregnancy in the women's life.[2] The earlier GDM was diagnosed prior to or at 20th week of gestation, while late GDM was detected during 24–28 weeks of the gestation period in the pregnant women.[3,4] Type 2 Diabetes Mellitus (T2DM) is one of the life time risk factor developed by the GDM women in the future and has the potential to be inherited to their offspring in future.[5] GDM women include advanced maternal age, previous history of GDM, family histories containing both T2DM and GDM as non-modified risk factors, while weight management, balanced diet, and regular physical activity are modified risk factors.[6] Obesity is considered as one of the major risk factors for the development of GDM in Saudi Arabia[7] as it alters the adipokine secretion and further leads to insulin resistance; which is definitely associated between diabetes and obesity.[8] The prevalence of obesity in Saudi women was ~41%.[9] The diagnosed GDM women will require lifestyle intervention modifications such as diet

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and physical activity, metformin as R_x and regular dietary supplements such as probiotics, inositol, and myoinositol that can promote weight loss or either improve insulin sensitivity.[10]

T2DM and GDM share the similar pathophysiological characteristics, precisely impaired insulin sensitivity and β-cell dysfunction.[11,12] Furthermore, GDM is believed to be equivalent to T2DM in terms of pathogenic mechanism, including insulin resistance and β-cell dysfunction.[13] The prevalence of T2DM is also parallelly growing in the Saudi population, and ethnicity plays an important role in contributing to each and every risk factor, even though their incidence varies among various population groups. The 19.6% of GDM prevalence was confirmed and documented in Saudi women.[7]

The diagnosis of GDM is confirmed via oral glucose tolerance test (OGTT), which was carried out between the later second and earlier third trimesters, ie, during 24–28 weeks of the gestation period in the pregnant women.[14] A 75-g OGTT diagnostic criteria by the American Diabetes Association (ADA) protocol will be followed by the outpatient clinic of the Department of Obstetrics and Gynecology at King Khalid University Hospitals (KKUH) in Saudi Arabia's capital city.

Sirtuin 1 (SIRT1) acts as an insulin sensor that helps regulate body weight and enhance insulin levels in skeletal muscle and adipose tissues. Additionally, it promotes insulin secretion and improves insulin sensitivity. With its histone deacetylase function on various substrates, SIRT1 plays a crucial role in regulating glucose and lipid metabolism. SIRT1 plays a critical role in regulating insulin signaling by enhancing insulin secretion in pancreatic β-cells.[15] Previous research has established that certain variants in the *SIRT1* gene play a role in developing visceral obesity, which can subsequently increase the risk of diabetes, precisely T2DM.[16–19] T2DM and GDM both have similar pathophysiological features, including peripheral insulin resistance and a lack of β-cell production in the pancreas. The *SIRT1* gene is crucial in maintaining glucose balance and insulin sensitivity throughout pregnancy, which can affect glucose tolerance and increase the risk of GDM.[20] The q21.3 locus on the 10th chromosome in *SIRT1* gene, which consists of 11 exons.[21]

Numerous global studies have been conducted and documented regarding *SIRT1* SNPs studied in different forms of diabetes related diseases.[15,22–27] However, limited studies have documented GDM women with SNPs present in *SIRT1* gene.[26,27] None of the studies addressed the combinational relationship between *SIRT1* gene and GDM women in Saudi Arabia. Furthermore, this study was designed to screen the rs4746720 and rs10823112 SNPs present in the *SIRT1* gene in Saudi women diseases with GDM.

Methods

Ethical Aspects

The ethical approval was received from the Institutional Review Board in the College of Medicine at King Saud University in 2023. All the pregnant women had signed the patient consent form, and next, women's anthropometric, baseline, family histories, and peripheral blood were collected. This study was carried out as per the norms of the Helsinki Declaration.

Sample Size Formula

In this study, a sample size calculation formula mentioned below was used to calculate the sample size for each group.

$$n = ((Z\alpha + Z\beta)2 * p * q * 2)/d^2$$

Where,
Z_α = Z value for α level
Z_β = Z value for β level
p = average percentage between two groups
q = 100-p
d = clinically meaningful difference between two groups.

Based on our previous work, we recruited 120 GDM and 120 non-GDM women using nMaster software to calculate the sample size. These GDM and non-GDM samples were adapted from Ali Khan et al[28] work, and this current study was designed to focus on the SNPs reported in *SIRT1* gene, which is different from earlier published work.

Involvement of Saudi Pregnant Women

The pregnant women were enrolled in the Department of Obstetrics and Gynecology at King Khalid University Hospital (KKUH). Altogether, 240 pregnant women were enrolled in this case-control study with equal number of cases and controls. The screening of GDM was confirmed in the 2nd trimester of the pregnancy, ie, between 24 and 28 weeks of gestation in the KKUH premises. The diagnosis of the GDM was confirmed using 75 g of OGTT. Prior to performance of 75 g OGTT test, all the pregnant women were recommended for overnight fasting or at least 8 hours after the sun set. Next day, fasting blood glucose (FBG) levels were measured, and then 75 g of OGTT test was carried out by collecting serum samples from 1st and 2nd hours to measure the glucose levels. The normal threshold levels for FBG, 1st and 2nd hours of OGTT levels were ≥5.1 mmol/L, ≥10.0 mmol/L, and ≥8.5mmol/L. During the screening of the glucose levels, controls (non-GDM) will be considered if the pregnant women had all the normal levels for three different types of serum analysis, ie, FBG, OGTT-1h, and OGTT-2h tests. If any one of the pregnant women meet or surpass the glucose levels for FBG, OGTT-1h and OGTT-2h tests during their pregnancy, then they are considered as GDM. The inclusion criteria for the selection of GDM women are based on screening at KKUH between 24 and 28 weeks of gestation age, Saudi nationality, and were not on medication diagnosed with other human diseases. The pregnant women who were already diabetes, non-Saudi nationality and confirmed GDM outside the KKUH were excluded from this study.[28]

Anthropometric Measurements and Sample Collection

Anthropometric measurements such as age, weight, BMI, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were started to be recorded on every visit to KKUH after the confirmation of their pregnancies. However, we recorded the anthropometric measurements after the completion of OGTT tests and diagnosed the women as either GDM or non-GDM. Based on documented protocols such as AAMI/ESH/ISO, HTN levels were used for this study.[29] Apart from this, 5 mL of peripheral blood was collected and bifurcated as serum (3 mL) and anticoagulant blood (2 mL). The serum sample was used for a panel of lipid profile analysis, and EDTA blood was used for measuring HbA1c levels and extraction of genomic DNA.

Biochemical Analysis

The biochemical panels of lipid profile and HbA1c assays was studied using with the peripheral blood samples. The values of FBG, PPBG, OGTT-1hr, and 2hrs of tests were obtained from medical records after enrolling the GDM and non-GDM women.

Molecular Sequencing Analysis

We used a Qiagen DNA isolation kit and collected peripheral blood in an EDTA vacutainer to extract the genomic DNA. The procedure was carried out with the RBC and WBC lysis and protein purification involving incubation and different forms of centrifugation to complete and observe 200 µL of genomic DNA from each sample. The NanoDrop spectrophotometer was used to quantify the 240 genomic DNAs to perform the DNA quantification and check the purity of genomic DNA, and finally, all the samples were converted into 20 µg/mL to perform the polymerase chain reaction (PCR) for screening of rs4746720 and rs10823112 SNPs. The amplification protocol was started with a 50 µL reaction consisting of Qiagen PCR master mix, a couple of primer sequences, genomic DNA, and a final volume of the reaction filled with purified water. We performed the PCR reaction in a normal thermal cycler (Applied Biosystems). Denaturation and extensions were optimized at 95°C and 72°C, while annealing was optimized at 64°C for both the SNPs. The performance of the PCR protocol was completed in 1.28 hrs, and complete details of SNPs used in this study have been shown in Table 1. To check the purity and quality of the genotyping for both SNPs, 2% agarose gel was prepared and run. All the PCR products were run on ethidium bromide-stained agarose gel using the UVI gel documentation system of the electrophoretic unit to confirm the presence of bands. Moreover, we purified the PCR samples and subjected them to Sanger sequencing analysis, a method often used in previous published work. We generated genomic data by analyzing chromatograms based on the presence of genotypes and colored peaks after receiving the FASTA, ABI, and PDF files. Figure 1 shows the presence of different genotypes and peaks among rs4746720 and rs10823112 SNPs after performing the Sanger sequencing analysis.

Table 1 Details of SIRT1 SNPs Included in This Study

Gene	rs Number	Location	Position	Substitution	Region	FWD Sequence	REV Sequence	Band Size
SIRT1	rs4746720	3'UTR +809	69346836	T-C	Exon-10	CAAAAAGCCATCGGAATGTT	TTAGCTGCCACAGTTTTGGA	185bp
SIRT1	rs10823112	IVS 8–1415	69340822	A-G (T-C)	Intron-9	CCATATCTCGTGGCTTTAGCA	CTGCACCACTGCACTCTAGC	240bp

Abbreviations: FWD Sequence, Forward Sequence; REV Sequence, Reverse Sequence.

Statistical Analysis

In this study, anthropometric, biochemical, clinical and genotype data were received from involved participants and used to perform the statistical analysis between GDM and non-GDM subjects. All the data from 240 pregnant women were recorded in an Excel sheet. The continuous variables were presented as mean ± SD and compared using a *t*-test between GDM and non-GDM. The categorical data were compared using the appropriate Chi-square/Fisher exact test. We used ANOVA to compare demographic parameters between SIRT1 genotypes and GDM and non-GDM women, using different BMI criteria. Since there were multiple comparisons, the Bonferroni corrections method was applied. Factors significantly associated with GDM ($p < 0.05$) in the univariate analysis were considered for the multivariable regression analysis. We used the penalized logistic regression analysis for the multivariable analysis to obtain reliable odds ratios (ORs) and 95% confidence limits (CIs). Many parameters, such as (weight, height, OGTT-1hr etc), despite being statistically significant in the univariate analysis, were not considered for the multivariable penalized logistic regression due to collinearity. This analysis was performed using SPSS version 25 and STATA version 16. SNP stats,[30] Haploview (Version 4.2), and generalized multifactorial dimensionality reduction (GMDR) model software were used in this study. Haplotype analysis and Linkage disequilibrium were studied using Haploview software. Along with the gene-gene interaction analysis, dendrogram, and graphical depletion methods, it was calculated using the GMDR model.

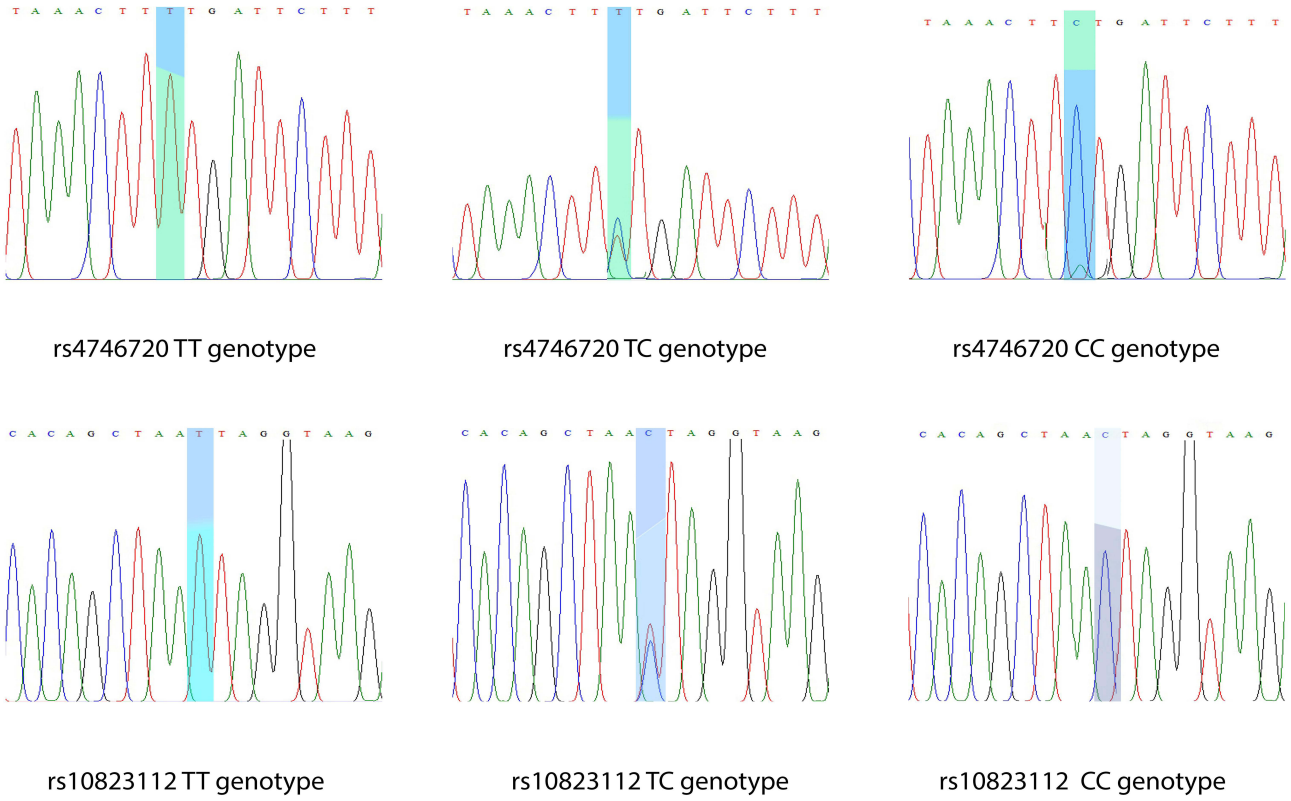

Figure 1 Identification of rs4746720 and rs10823112 SNPs present in GDM and non-GDM women using Sanger sequencing analysis.

Demographic data, genotype, and allele frequencies using (GDM vs non-GDM and obesity vs non-obesity) were also studied. Statistical significance was defined as $p < 0.05$.

Results

Basic Information

In this study, a total of 240 pregnant Saudi women were recruited based on positive and negative OGTT levels during their second trimester of pregnancy. The 120 pregnant women with elevated OGTT levels were confirmed as GDM, and non-GDM women were confirmed after obtaining normal glucose levels. However, all the GDM women were diabetic for the first time during their initial and multiple pregnancies.

Demographic Features of GDM and Non-GDM Women

Table 2 of this study represents the demographic features of GDM and non-GDM women and involves a comprehensive overview of 240 pregnant women's clinical, biochemical, molecular and anthropometric data. In this study, GDM women were older than non-GDM women ($p = 0.001$) after comparing the mean (± SD) ages (31.92 ± 5.27 vs 28.48 ± 6.10). The

Table 2 Clinical and Demographical Parameters Studied Between GDM and Non-GDM Women(s) as a Case-Control Study

Covariant(s)	GDM (n = 120)	Non-GDM (n = 120)	Mean Difference (95% Cis)	P value
	Mean ± SD			
Age (Years)	31.92 ± 5.27	28.48 ± 6.10	3.44 (1.99–4.89)	0.001
Weight (kgs)	77.40 ± 12.94	72.95 ± 12.04	4.45 (1.28–7.63)	0.006
Height (cms)	158.64 ± 6.49	157.97 ± 5.25	0.68 (−0.82, 2.17)	0.380
BMI (Kg/m^2)	30.65 ± 4.81	29.16 ± 4.53	1.49 (0.30–2.68)	0.01
SBP (mmHg)	131.97 ± 11.22	120.58 ± 5.29	11.38 (9.15–13.61)	<0.0001
DBP (mmHg)	83.10 ± 7.59	78.51 ± 4.44	4.59 (3.01–6.17)	<0.0001
BP (n, %)	64 (53.3)	02 (1.7)	51.6 (42–62)	<0.0001
FBG (mmol/L)	5.55 ± 1.58	4.53 ± 0.58	1.01 (0.71–1.31)	<0.0001
PPBG (mmol/L)	8.15 ± 15.28	5.07 ± 0.92	1.79 (1.30–2.29)	<0.0001
OGTT-1hr (mmol/L)	10.83 ± 1.97	7.08 ± 1.36	3.75 (3.32–4.18)	<0.0001
OGTT-2hr (mmol/L)	9.40 ± 1.67	6.50 ± 1.44	2.89 (2.50–3.29)	<0.0001
HbA1c (%)	5.58 ± 0.48	5.22 ± 0.34	0.36 (0.26–0.47)	<0.0001
TG (mmol/L)	2.18 ± 1.22	2.20 ± 1.92	−0.03 (−0.44, 0.38)	0.923
TC (mmol/L)	5.57 ± 1.11	5.76 ± 1.25	−0.19 (−0.49, 0.11)	0.213
HDLc (mmol/L)	0.68 ± 0.33	0.98 ± 0.41	−0.30 (−0.39,-0.20)	<0.0001
LDLc (mmol/L)	3.86 ± 0.82	3.93 ± 0.97	−0.07 (−0.30, 0.16)	0.546
Medication (R_x)	110 (91.67%)/10 (8.33%)	–	–	–
T2DM Family History	57 (47.5%)	48 (40%)	7.5 (−6, 208)	0.24
GDM Family History	28 (23.3%)	24 (20%)	3.3 (−8, 14.5)	0.53

Abbreviations: BMI, Body Mass Index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; FBG, Fasting Blood Glucose; PPBG, Post Prandial Blood Glucose; OGTT, Oral Glucose Tolerance Test; HbA1c, Glycated Hemoglobin; TG, Triglycerides; TC, Total Cholesterol; HDLc, High Density Lipoprotein Cholesterol; LDLc, Low Density Lipoprotein Cholesterol; T2DM, Type 2 Diabetes Mellitus and GDM, Gestational Diabetes Mellitus.

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GDM women were in the obese category (30.65 ± 4.81), while non-GDM (29.16 ± 4.53) were under the overweight category (p = 0.01). Subsequently, weight was also reflected in the BMI in both GDM (77.40 ± 12.94) and non-GDM women (72.95 ± 12.04) and was found to be significantly associated (p = 0.006). Both the HTN levels, ie, SBP (131.97 ± 11.22 vs 120.58 ± 5.29) and DBP (83.10 ± 7.59 vs 78.51 ± 4.44) had elevated levels in GDM women and were associated (p < 0.0001). In 53.3% of GDM women were found to have blood pressure (BP) and in non-GDM women, only 1.7% of were confirmed and showed the strong association (p < 0.0010). Different forms of glucose levels such as FBG (5.55 ± 1.58 vs 4.53 ± 0.58), OGTT-1hr (10.83 ± 1.97 vs 7.08 ± 1.36), OGTT-2hr (9.40 ± 1.67 vs 6.50 ± 1.44), PPBG (8.15 ± 15.28 vs 5.07 ± 0.92) and HbA1c levels (5.58 ± 0.48 vs 5.22 ± 0.34) were elevated in GDM women and associated when studied and compared with non-GDM women (p < 0.0001). Among the lipid profile parameters, there were difference in the levels of HDLc between GDM and non-GDM women (0.68 ± 0.33 vs 0.98 ± 0.41; p < 0.001) and other parameters, such as TC (5.57 ± 1.11 vs 5.76 ± 1.25; p = 0.213), TG (2.18 ± 1.22 vs 2.20 ± 1.92) and LDLc levels (3.86 ± 0.82 vs 3.93 ± 0.97) were not consistent (p > 0.05). The family histories of GDM (23.3% vs 20%; p = 0.001) and T2DM (47.5% vs 40%; p < 0.001) in GDM and non-GDM women were found to be significantly associated (p < 0.05).

Penalized Logistic Regression Analysis

On the adjusted penalized logistic regression analysis (Table 3), when there was one unit change in FBG, the risk of having GDM was significantly 3.05 (95% CI: 1.06–8.78) times higher compared with non-GDM (p = 0.04). Similarly, when there was unit change in OGTT-2hr, the risk of having GDM was about 9 (95% CI: 3.48–22.9) times significantly higher (p < 0.001). However, lowering the HDLc values provides 93% (95% CI: 1–46%) less risk of having GDM than

Table 3 Comparison of Penalized Logistic Regression of Clinical and Demographic Parameters Between GDM and Non-GDM

Covariant(s)	Univariate		Multivariate	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Age	1.10 (1.06–1.16)	<0.001	1.08 (0.97–1.20)	0.17
BMI	1.07 (1.01–1.13)	0.02	1.02 (0.90–1.17)	0.72
FBG	4.64 (2.80–7.67)	<0.001	3.05 (1.06–8.78)	0.04
PPBG	1.76 (1.46–2.13)	<0.001	1.35 (0.96–1.91)	0.09
OGTT-1hr	3.35 (2.52–4.45)	<0.001		
OGTT-2hr	4.27 (2.93–6.21)	<0.001	8.92 (3.48–22.9)	<0.001
HbA1c	11.25 (4.85–26.13)	<0.001	1.81 (0.25–13.32)	0.56
TG	0.99 (0.85–1.16)	0.90		
TC	0.87 (0.87–1.08)	0.22		
HDLc	0.11 (0.05–0.25)	<0.001	0.07 (0.01–0.46)	0.01
LDLc	0.91 (0.69–1.21)	0.53		
Family History				
T2DM	1.36 (0.81–2.26)	0.24		
GDM	1.22 (0.66–2.25)	0.53		
BP	54.1 (14.7–199.1)	<0.001	41.1 (4.5–375.9)	0.001

Abbreviations: BMI, Body Mass Index; FBG, Fasting Blood Glucose; PPBG, Post Prandial Blood Glucose; OGTT, Oral Glucose Tolerance Test; HbA1c, Glycated Hemoglobin; TG, Triglycerides; TC, Total Cholesterol; HDLc, High Density Lipoprotein Cholesterol; LDLc, Low Density Lipoprotein Cholesterol; BP, Blood Pressure.

non-GDM (p = 0.01). The women who had a history of BP during pregnancy had 41 (95% CI: 4.1–376) times significantly higher risk of getting GDM compared who do not had BP during pregnancy (p = 0.001).

Population Genetical Association Analysis in rs4746720 and rs10823112 SNPs

The details of alleles and genotype frequencies present in GDM and non-GDM women among rs4746720 and rs10823112 SNPs in *SIRT1* gene was shown in Table 4. None of the allele frequencies were found to be positively associated either in rs4746720 (T vs C: OR-0.95 [95% CI: 0.65–1.39]; p = 0.78) and rs10823112 SNPs (T vs C: OR-1.43 [95% CI: 0.95–2.17]; p = 0.07). The allele frequencies of T and C in GDM and non-GDM women were 0.57%, 0.43% and 0.56%, 0.44% in rs4746720 SNP. For rs10823112 SNP, the allele frequencies of T and C were 0.67%, 0.33% in GDM and 0.74%, 0.26% in non-GDM women. The genotype frequencies for rs4646720 SNP in GDM and non-GDM women were found to be 32.5%, 49.2%, 18.3% and 23.3%, 65%, 11.7% in TT, TC, and CC genotypes. In this study, dominant and co-dominant models (TC+CC vs TT: OR-1.80 [95% CI: 1.12–2.89]; p = 0.01 and TT+CC vs TC: OR-1.92 [95% CI: 1.11–3.33]; p = 0.01) were associated (p < 0.05); however, recessive model was not associated (TT+TC vs CC: OR-0.59 [95% CI: 0.26–1.28]; p = 0.148). Additionally, genotypes were also not associated (TC vs TT: OR-0.54 [95% CI: 0.30–0.98]; p = 0.04 and CC vs TT: OR-0.48 [95% CI: 1.12–2.89]; p = 0.053). The TT, TC, CC genotypes of rs10823112 SNP consist of 45.8%, 41.7%, 12.5% in GDM women and 55.8%, 36.7%, 7.5% in non-GDM women. There was no significant association was documented either in genotypes (TC vs TT: OR-1.38 [95% CI: 0.81–2.38]; p = 0.24; CC vs TT: OR-0.68 [95% CI: 0.27–1.72]; p = 0.41) or different forms of genetic models (TC+CC vs TT: OR-1.49 [95% CI: 0.90–2.49]; p = 0.121; TT+CC vs TC: OR-0.81 [95% CI: 0.47–1.40]; p = 0.43; TT+TC vs CC: OR-0.57 [95% CI: 0.21–1.45]; p = −0.20).

Table 4 Probable Association Between Genotype and Allele Frequencies Present in GDM and Non-GDM Women Studied in SIRT1 Gene SNPs

Gene (rs Number)	Genotypes	GDM (n = 120)		Non-GDM (n = 120)		OR (95% CI)	p-value
		n	%	n	%		
SIRT1 (rs 4746720)	TT	39	32.5	28	23.3	1.00	
	TC	59	49.2	78	65.0	0.54 (0.30–0.98)	0.04
	CC	22	18.3	14	11.7	0.48 (0.22–1.02)	0.05
	TC+CC vs TT	81	67.5	92	76.6	1.80 (1.12–2.89)	0.01
	TT+CC vs TC	61	50.8	42	35.0	1.92 (1.11–3.33)	0.01
	TT+TC vs CC	98	81.6	106	83.3	0.59 (0.26–1.28)	0.15
	T allele	137	57.0	134	56.0	1.00	
	C allele	103	43.0	106	44.0	0.95 (0.65–1.39)	0.78
SIRT1 (rs 10823112)	TT	55	45.8	67	55.8	1.00	
	TC	50	41.7	44	36.7	1.38 (0.81–2.38)	0.24
	CC	15	12.5	9	7.5	0.68 (0.27–1.72)	0.41
	TC+CC vs TT	65	54.2	53	44.2	1.49 (0.90–2.49)	0.12
	TT+CC vs TC	70	58.3	76	63.3	0.81 (0.47–1.40)	0.43
	TT+TC vs CC	105	87.5	111	92.5	0.57 (0.21–1.45)	0.20
	T allele	160	67.0	178	74.0	1.00	
	C allele	80	33.0	62	26.0	1.43 (0.95–2.17)	0.07

Abbreviations: n, total numbers; %, Percentages.

ANOVA Analysis Studies

Table 5 in this study consists of ANOVA analysis carried out between rs4746720 and rs10823112 SNPs in GDM women. TT, TC, CC genotypes in rs4746720 and rs10823112 SNPs along with 15 dependent covariates and the study results have found that the rs4746720 SNP genotypes were linked to levels of FBG ($p = 0.002$), PPBG ($p = 0.0001$), OGTT-2hr ($p = 0.02$) and HDLc ($p = 0.0003$). The rs10823112 SNP genotypes were connected to PPBG ($p = 0.0001$), HbA1c ($p = 0.001$) and HDLc ($p = 0.01$) levels were associated ($p < 0.05$). Most importantly, PPBG and HDLc levels ($p < 0.05$).

Genetical and Statistical Analysis in GDM Women with and without Obesity

The total of 120 GDM were categorized as 56.7% (n = 68) as obese GDM women and 43.3% (n = 52) as non-obese GDM women. Table 6 consists of genotype and allele frequencies for obese and non-obese women among GDM women along with the statistical association. For rs4746720 SNP, 32.4%, 50%, 17.6% of TT, TC, CC genotypes were found in obese GDM women and 32.7%, 48.1%, 19.2% of non-obese GDM women. The allele frequencies for T and C groups for obese and non-obese subgroups were found to be 57.35%, 42.65%, and 56.73%, 43.27% in GDM women. None of the genotypes, (TC vs TT: OR-1.05 [95% CI: 0.46–2.38]; $p = 0.91$; CC vs TT: OR-0.93 [95% CI: 0.32–2.65]; $p = 0.89$) dominant, co-dominant and recessive models (TC+CC vs TT: OR-1.02 [95% CI: 0.47–2.19]; $p = 0.97$; TT+CC vs TC: OR-0.93 [95% CI: 0.45–1.91]; $p = 0.83$; TT+TC vs CC: OR-1.11 [95% CI: 0.44–2.82]; $p = 0.82$) and allele frequencies (C vs T: OR-0.97 [95% CI: 0.58–1.63]; $p = 0.92$) showed the association between obese and non-obese subjects in rs4746720 SNP. Next, the rs10823112 SNP was also studied in obese and non-obese subjects in the GDM women. The genotype frequencies for obese and non-obese subjects were found to be 44.1% (n = 30), 41.2% (n = 28), 14.7% (n = 10) in TT, TC, CC genotypes and 48.1% (n = 25), 42.3% (n = 22), 9.6% (n = 05) in the GDM women. Additionally, the allele frequencies for obese and non-obese women were found to be 67.41%, 35.29% and 69.23%, 30.77% in T and C alleles in rs10823112 SNP. The statistical analysis showed the non-significant association in genotype models (TC vs TT: OR-1.06

Table 5 ANOVA Analysis Studied Between SIRT1 Genotypes and Dependent Factors in GDM Women

	***SIRT1* (rs4746720)**				***SIRT1* (rs10823112)**			
	TT (n = 39)	**TC (n = 59)**	**CC (n = 22)**	**p value**	**TT (n = 55)**	**TC (n = 50)**	**CC (n = 15)**	**p value**
Age	32.00 ± 4.98	31.80 ± 5.50	32.09 ± 5.38	0.79	32.53 ± 5.17	31.32 ± 5.39	31.67 ± 5.30	0.95
Weight	78.44 ± 14.49	76.47 ± 12.23	78.08 ± 12.28	0.47	77.52 ± 13.55	76.10 ± 10.95	81.33 ± 16.51	0.10
BMI	30.46 ± 4.92	30.75 ± 4.73	30.73 ± 5.07	0.91	30.43 ± 4.74	30.27 ±4.22	32.74 ± 6.53	0.09
SBP	131.87 ± 11.62	132.76 ± 11.22	130.00 ± 10.78	0.92	132.93 ± 11.09	129.88 ± 11.28	135.40 ± 10.95	0.98
DBP	82.28 ± 8.44	84.22 ± 6.83	81.55 ± 7.87	0.34	83.15 ± 7.66	82.00 ± 7.75	86.60 ± 6.02	0.52
FBG	5.68 ± 1.78	5.68 ± 1.62	4.95 ± 0.86	0.002	5.64 ± 1.66	5.45 ± 1.63	5.50 ± 1.16	0.28
PPBG	10.77 ± 26.61	7.02 ± 2.28	6.55 ± 2.80	0.0001	9.46 ± 22.47	6.94 ± 2.14	7.41 ± 2.59	0.0001
OGTT-1hr	10.73 ± 2.19	10.93 ± 1.99	10.70 ± 1.52	0.19	10.92 ± 2.01	10.61 ± 1.85	11.22 ± 2.25	0.62
OGTT-2hr	9.40 ± 1.79	9.30 ± 1.78	9.66 ± 1.04	0.02	9.33 ± 1.60	9.37 ± 1.58	9.75 ± 2.18	0.68
HbA1c	5.68 ± 0.55	5.58 ± 0.44	5.41 ± 0.44	0.26	5.61 ± 0.53	5.56 ± 0.50	5.56 ± 0.21	0.001
TG	2.19 ± 1.37	2.21 ± 1.20	2.06 ± 1.04	0.35	2.17 ± 1.27	2.12 ± 1.23	2.38 ± 1.06	0.71
TC	5.60 ± 0.95	5.48 ± 1.11	5.78 ± 1.38	0.14	5.44 ± 1.11	5.71 ± 1.16	5.58 ± 0.96	0.69
HDLc	0.65 ± 0.26	0.64 ± 0.28	0.85 ± 0.50	0.0003	0.66 ± 0.35	0.72 ± 0.35	0.64 ± 0.17	0.01
LDLc	3.84 ± 0.74	3.82 ± 0.79	3.99 ± 1.02	0.20	3.77 ± 0.72	3.95 ± 0.94	3.86 ± 0.73	0.13

Abbreviations: BMI, Body Mass Index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; FBG, Fasting Blood Glucose; PPBG, Post Prandial Blood Glucose; OGTT, Oral Glucose Tolerance Test; HbA1c, Glycated Hemoglobin; TG, Triglycerides; TC, Total Cholesterol; HDLc, High Density Lipoprotein Cholesterol; LDLc, Low Density Lipoprotein Cholesterol.

Table 6 Possible Association in GDM Women with and without Obesity in SIRT1 Gene SNPs

Gene (rs Number)	Genotypes	Obese GDM (n = 68)		Non-Obese GDM (n = 52)		OR (95% CI)	p-value
		n	%	n	%		
SIRT1 (rs 4746720)	TT	22	32.4	17	32.7	1.00	
	TC	34	50.0	25	48.1	1.05 (0.46–2.38)	0.91
	CC	12	17.6	10	19.2	0.93 (0.32–2.65)	0.89
	TC+CC vs TT	46	67.6	35	67.3	1.02 (0.47–2.19)	0.97
	TT+CC vs TC	34	50.0	27	51.9	0.93 (0.45–1.91)	0.83
	TT+TC vs CC	56	82.4	42	80.8	1.11 (0.44–2.82)	0.82
	T allele	78	57.4	59	56.7	1.00	
	C allele	58	42.6	45	43.3	0.97 (0.58–1.63)	0.92
SIRT1 (rs 10823112)	TT	30	44.1	25	48.1	1.00	
	TC	28	41.2	22	42.3	1.06 (0.49–2.29)	0.88
	CC	10	14.7	05	9.6	1.67 (0.50–5.52)	0.40
	TC+CC vs TT	38	44.9	27	51.9	1.17 (0.57–2.42)	0.67
	TT+CC vs TC	40	58.8	30	57.7	1.05 (0.50–2.18)	0.90
	TT+TC vs CC	58	85.3	47	90.4	0.62 (0.20–1.93)	0.40
	T allele	88	64.7	72	69.2	1.00	
	C allele	48	35.3	32	30.8	1.23 (0.71–2.12)	0.46

Abbreviations: n, total numbers; %, Percentages.

[95% CI: 0.49–2.29]; p = 0.88; CC vs TT: OR-1.67 [95% CI: 0.50–5.52]; p = 0.40), genetic models (TC+CC vs TT: OR-1.17 [95% CI: 0.57–2.42]; p = 0.67; TT+CC vs TC: OR-1.05 [95% CI: 0.50–2.18]; p = 0.90; TT+TC vs CC: OR-0.62 [95% CI: 0.20–1.93]; p = 0.40) and allele frequencies (C vs T: OR-1.23 [95% CI: 0.71–2.12]; p = 0.46).

ANOVA Analysis with Demographic Features of Obesity Among Pregnant Women

In this study, the GDM women consist of 13.3% (n = 16), with normal BMI levels, 30% (n = 36) overweight women, 39.2% (n = 47) obese, 14.2% (n = 17) morbid obese-I and 3.3% (n = 04) morbid obese-II women, while in non-GDM group, 17.5% (n = 21) were having normal BMI levels, 38.3% (n = 46) were overweight, 35.3% (n = 43) were obese, 6.7% (n = 08) were morbid obese-I, and 1.7% (n = 02) were morbid obese-II, groups. The demographic features are involved with age, weight, BMI, FBG, PPBG, OGTT-1hr, OGTT-2hr and HbA1c levels as shown in Table 7. When ANOVA analysis was studied in different forms of obesity levels in GDM women, Weight (p = 0.0002), BMI (p = 0.00001), FBG (p = 0.0001), PPBG (p = 0.00001) and HbA1c (p = 0.0001) were associated and in non-GDM women, Age (p = 0.01), Weight (p = 0.0001), BMI (p = 0.00001), PPBG (p = 0.02) and OGTT-2hr (0.008). Overall, weight and BMI were commonly associated in GDM and non-GDM women with obesity.

Haplotype Analysis for SIRT1 SNPs

Haplotype analysis was studied with T and C alleles present in rs4746720 and rs10823112 SNPs with the obtained combinations of TT, CT, TC, CC alleles. There was no genetic association present in TT (p = 0.00), CT (p = 0.57), TC (p = 0.54) and CC (p = 0.3) between GDM, and non-GDM groups. All the details were shown in Table 8.

Table 7 Calculation of Demographic Features in GDM and Non-GDM Women-Using BMI Criteria

Obesity in GDM Women	Age	Weight	BMI	FBG	PPBG	OGTT-1	OGTT-2	HbA1c
Normal levels (n = 16)	31.25 ± 4.43	61.83 ± 3.66	23.69 ± 0.83	4.89 ± 0.52	5.80 ± 2.62	11.05 ± 1.27	9.42 ±1.27	5.56 ± 0.41
Overweight (n = 36)	31.53 ± 5.51	69.47 ± 6.19	27.39 ± 1.57	5.88 ± 1.91	7.01 ± 2.09	10.59 ± 2.09	9.36 ± 1.60	5.44 ± 0.31
Obese-I (n = 47)	31.34 ± 5.08	81.14 ± 10.12	32.22 ± 1.37	5.61 ± 1.67	6.59 ± 2.78	10.79 ± 1.91	9.49 ± 1.81	5.68 ±0.52
Obese-II (n = 17)	34.76 ± 5.48	93.18 ± 4.71	36.91 ± 1.28	5.29 ± 0.97	16.78 ± 40.02	10.87 ± 2.36	9.09 ± 1.67	5.68 ± 0.70
Obese-III (n = 04)	32.75 ± 6.02	100.25 ± 8.18	42.88 ± 1.93	5.50 ± 1.94	9.48 ± 1.06	12.20 ± 2.43	9.90 ± 2.53	5.43 ± 0.10
P values	0.88	0.0002	0.00001	0.0001	0.00001	0.20	0.42	0.0001
Obesity in Non-GDM Women	**Age**	**Weight**	**BMI**	**FBG**	**PPBG**	**OGTT-1**	**OGTT-2**	**HbA1c**
Normal levels (n = 21)	26.29 ± 5.52	56.54 ± 7.26	22.39 ± 1.98	4.66 ± 0.46	5.26 ± 1.03	7.36 ± 1.31	6.40 ± 1.24	5.40 ± 0.28
Overweight (n = 46)	28.07 ± 5.80	69.31 ± 4.83	27.72 ± 1.25	4.51 ± 0.67	5.05 ± 0.93	7.11 ± 1.38	6.99 ± 1.09	5.23 ± 0.30
Obese-I (n = 43)	29.16 ± 6.12	79.99 ± 5.80	32.01 ± 1.56	4.52 ± 0.54	4.91 ± 0.70	6.91 ± 1.46	5.94 ± 1.61	5.12 ± 0.36
Obese-II (n = 08)	34.38 ± 6.07	92.25 ± 7.15	36.86 ± 1.36	4.50 ± 0.50	5.35 ± 1.05	6.95 ± 0.99	6.71 ± 1.83	5.23 ± 0.46
Obese-III (n = 02)	22.50 ± 0.71	100.10 ± 22.77	41.35 ± 1.91	4.00 ± 1.35	5.75 ± 2.76	7.30 ± 0.14	7.50 ± 1.41	5.25 ± 0.07
P values	0.01	0.0001	0.00001	0.16	0.02	0.31	0.008	0.20

Abbreviations: BMI, Body Mass Index; FBG, Fasting Blood Glucose; PPBG, Postprandial Blood Glucose; OGTT, Oral Glucose tolerance tests for 1st and 2nd hours; HbA1c, Glycated hemoglobin.

Table 8 Haplotype Analysis Investigated in GDM and Non-GDM Women

S. No	rs4746720	rs10823112	Frequency	OR (95% CI)	P value
1	T	T	0.405	1.00	–
2	C	T	0.2992	0.85 (0.48–1.49)	0.57
3	T	C	0.1596	1.24 (0.62–2.51)	0.54
4	C	C	0.1362	1.40 (0.75–2.64)	0.3

Linkage Disequilibrium Analysis Studies in SIRT1 Gene

Table 9 in this study has documented the LD analysis, and the combination of GDM women was not associated and had no role ($p = 0.13$), while in the non-GDM women, there was a strong significant association studied between rs10823112 and rs4746720 SNPs ($p < 0.05$). Additionally, Figure 2 showed the association with GDM and a strong association with non-GDM women. This indicates the rs4746720 and rs10823112 SNPs have a role in non-GDM women.

Analysis for GMDR Models

GMDR models were studied in this study in different forms, such as gene-gene interaction analysis (Table 10), and different predicted models (Figure 3) such as dendrogram analysis and a graphical depletion model in GDM and non-

Table 9 Linkage Disequilibrium Analysis Studies in SIRT1 Gene SNPs

Pregnant Women	L1	L2	D′	r^2
GDM cases	rs10823112	rs4746720	0.079	0.13
Non-GDM	rs10823112	rs4746720	0.009	0.0

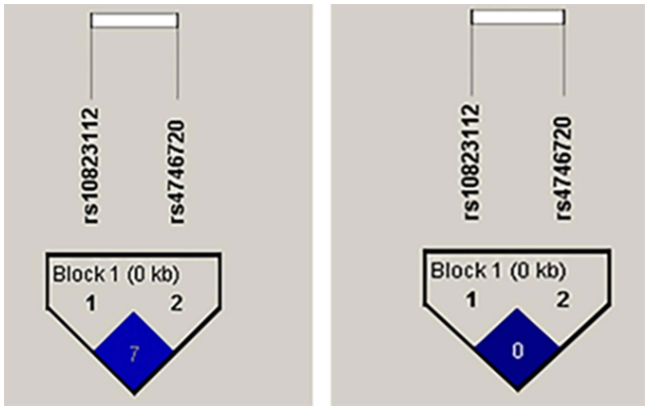

Figure 2 Linkage disequilibrium analysis studies in *SIRT1* SNPs in GDM and non-GDM women.

GDM women with rs4746720 and rs10823112 SNPs in *SIRT1* gene. The gene-gene interaction analysis confirmed a negative association with the first model of rs4746720 SNP ($p = 0.06$) and a positive association with the second model of the combination of rs4746720 and rs10823112 SNPs ($p = 0.0038$). The CVCs for the first and second models were 9/10 and 10/10. Blue-colored flat dendrograms were present and represented the redundancies. In the graphical depletions, high risks are present in dark cells and low risks are present in the lighter cells. However, blank cells indicate the absence of genotype data. The graphical depletion model confirmed the nominal risk only with the rs4746720 SNP. The overall analysis of GMDR confirmed that the nominal association risk is present in the GDM women.

Discussion

Diabetes and obesity are becoming highly frequent in Saudi Arabia due to their high prevalence, and GDM is also increasing concurrently in the sub-category of diabetes mellitus. Considering family history, BMI values, lifestyle habits and environmental factors will contribute to future complications. The prevalence of GDM in the Saudi Arabia has been ripening gradually[31] by 2–3 folds ranging from 8.9% to 53.4%. However, Alfadhli et al[32] studies have addressed the increase in the prevalence of GDM in Saudi Arabia, which is mainly due to adoption of new diagnostic criteria for screening of GDM by IADPSG group.[32] This study included 75 g of 2-hour OGTT values, as recommended by ADA criteria. This study was carried out from a single tertiary care center, rather than multiple tertiary care. The aim of this study was to investigate the molecular screening for rs4746720 and rs10823112 SNPs present in women diagnosed with GDM and non-GDM women based on OGTT levels in the Saudi Arabia. The study results confirmed the genetic association with ANOVA analysis, LD analysis and GMDR models ($p < 0.05$) including rs4746720 and rs10823112 SNPs in *SIRT1* gene.

Global wide studies were carried out with different SNPs studied in *SIRT1* gene in human diseases. *SIRT1* gene is associated with T2DM,[23,33–46] various forms of diabetes[24,34,36,38,43,45–54] and other human diseases such as obesity,[16,17,19,50,55–64] chronic diseases,[65,66] CAD,[35,49,67–74] rheumatoid,[75,76] sclerosis,[77,78] and female diseases such as pre-eclampsia,[79] endometriosis,[80] and female infertility.[81] Apart from humans, *SIRT1* SNPs were also studied in cattle's/sheep's.[82–89] Additionally, meta-analysis studies were also documented in *SIRT1* SNPs.[90–93] The serum levels were also studied in obesity patients with NAFLD,[94] Parkinson's disease,[95] and other human diseases.[96–100] In a Saudi population, Kaabi et al[41] have performed the molecular studies in rs12778366 and rs3758391 SNPs in *SIRT1* gene and showed the

Table 10 Gene-Gene Interaction Analysis Used in Analyzing the GDM Risk in SIRT1 Gene SNPs

Model No	Combinations of *SIRT1* Gene SNPs (n = 02)	Training Accuracy	Testing Accuracy	CVC	Total Sensitivity	Total Specificity	Kappa	F-Measure	χ^2	OR (95% CI)	P value
1	rs4746720	0.5792	0.5500	9/10	0.5093	0.6667	0.1759	0.5528	3.4275	2.075 (0.9536–4.5171)	0.0641
2	rs4746720+rs10823112	0.6292	0.6167	10/10	0.6019	0.6759	0.2778	0.6250	8.3793	3.1528 (1.4331–6.9362)	0.0038

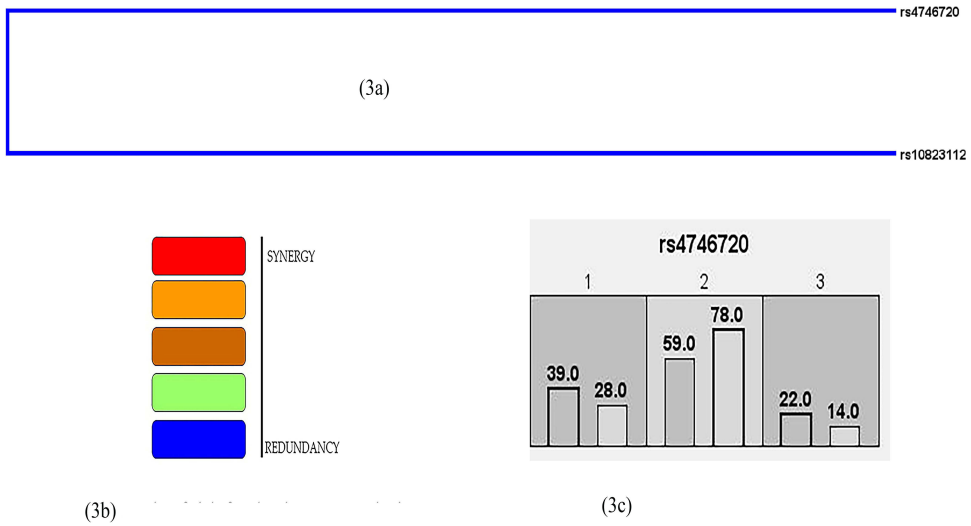

Figure 3 Precited models presented in graphical and depletion methods analyzed using GMDR model in GDM women. (**a**) Dendrogram analysis (bold number specifies the rs numbers used in this study ie, rs4746720 and rs10823112), (**b**) mode of risk for dendrogram analysis, (**c**) graphical depletion model (bold number rs4746720 SNP indicates that it was involved this model).

negative association. In this study, rs4746720 and rs10823112 SNPs were studied in pregnant (GDM and non-GDM) women, and the study results are not associated with other ethnicities because, till now, there are no molecular studies documented between GDM and *SIRT1* SNPs.

This study examined many characteristics of glucose levels, which demonstrated a favorable correlation with ANOVA analysis (Table 5 and Table 7), LD analysis (Table 9), and gene-gene interaction analysis (Table 10). This study analysis suggests that while *SIRT1* SNPs may not influence genotyping analysis, they do impact glucose levels, indicating a relationship between GDM and the *SIRT1* gene.

SIRT1 activation influences glucose/lipid metabolism in diabetes patients, as shown in Figure 4. It helps to preserve pancreatic β-cells and enhance insulin secretion while also promoting lipid mobilization in adipose tissue, glucose uptake

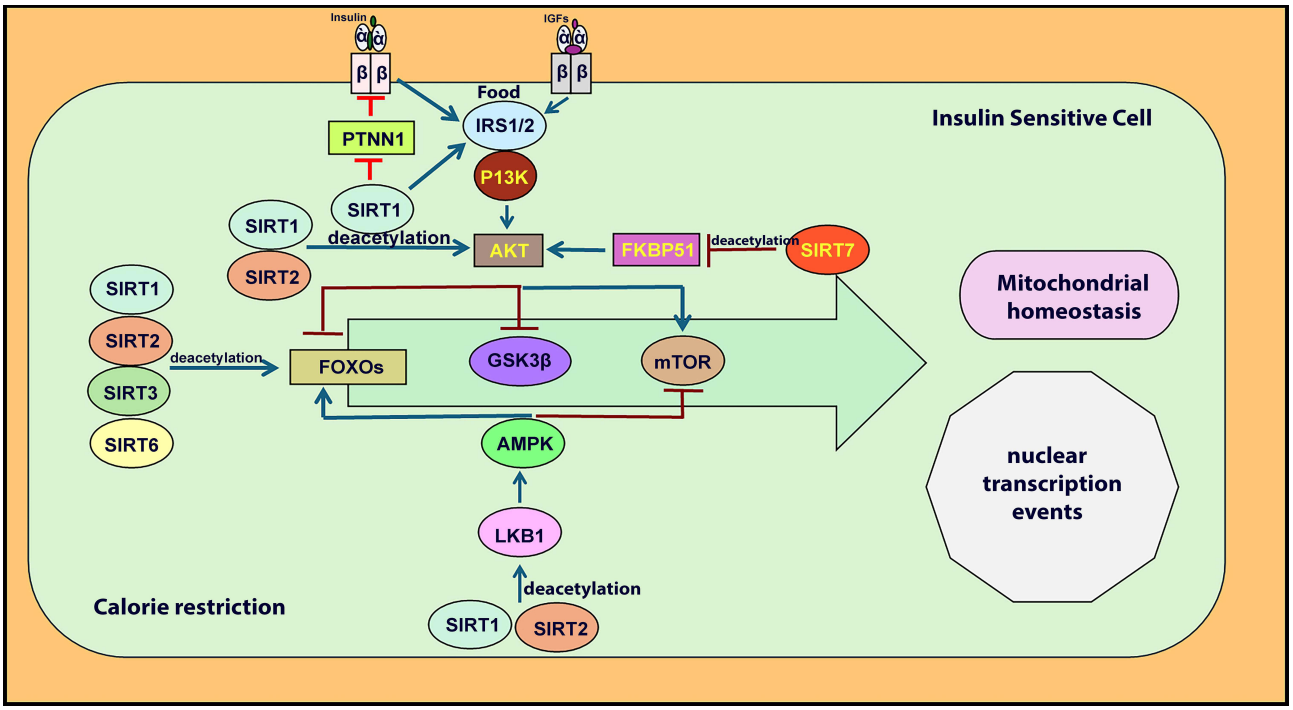

Figure 4 Role of sirtuins in insulin signaling pathways.

in skeletal muscle, and mitochondrial biogenesis. In addition, it enhances insulin sensitivity in the liver and muscle. SIRT1 effectively suppresses Uncoupling Protein 2 from working in the pancreas by interacting with FOXO. This causes insulin production to growth and promotes the survival of β cells.[101–103] In a study by Lee et al, it was shown that SIRT1 has the ability to inhibit NF-κB activation, thereby providing protection to β cells against oxidative damage and cytokines. When SIRT1 is overexpressed, it suppresses cytokines from killing cells and halts the production of nitric oxide synthase (iNOS). Through deacetylating the NF-κB p65 unit, SIRT1 effectively decreases iNOS production.[104] Previous study discovered that increasing the expression of SIRT1 in pancreatic β-cells of transgenic mice resulted in enhanced glucose and release insulin.[105]

However, maintaining normal NAD^+ production restores glucose-sensitive insulin secretion and improves glucose tolerance in old BESTO animals. SIRT1 protein expression is decreased among diabetic patients. Low SIRT1 levels contribute to muscular insulin resistance. However, it has been observed that SIRT1 can interact with PI3K adaptor subunit p85 without insulin. This interaction allows SIRT1 to effectively regulate insulin signaling in skeletal muscle cells, even at normal insulin concentrations. In addition, resveratrol, which turns on SIRT1, can protect muscle cells from insulin resistance triggered by TNF-α or prolonged hyperinsulinemia.[106] However, we have revised and created Figure 4 from Ulu et al[106] studies, translated from Turkish to English language.

Family history has an important element in human diseases. In the current era, GDM is increasing in tandem with the growing prevalence of T2DM and obesity.[107] Diabetes is among the designated conditions that manifest with particular combinations within familial circumstances. Apart from family and self-histories, undesirable habits designate our future quality of life, whether it will be disease-free or an unhealthy or medication lifestyle. In this study, 23.3% of the GDM women had a family history of GDM and 47.5% had a family history of T2DM, while 40% had a family history of T2DM and 20% of family history with GDM is present in non-GDM women. Thus, the GDM women may have future complications, especially for developing T2DM between 5 and 10 years of their life. Furthermore, consanguinity is also playing an important role in GDM women of Saudi Arabia. The accurate prevalence of consanguinity is not documented in the recent decade, although the previously documented prevalence was around 40–50% in Saudi Arabia.[108–114] The impact of consanguineous marriages will lead to genetic disorders in the future in all the family members and when it comes in married female cousin will leads to direct or indirect reproductive issues which include multifactorial disorders such as diabetes, asthma, cancers, obesity, mental retardation, epilepsy and infections. The married women may face fertility and pregnancy complications, as pre-eclampsia, eclampsia, GDM, and miscarriages can also occur. Majorly, the children born to consanguineous parents will be affected with genetic disorders.[115] One of the limitations in this study was not documenting the details of consanguinity in the pregnant women.

In this study, 91.7% of GDM women were on a diet, and 8.3% of them were on insulin. None of the GDM women were on medication. Carbohydrates are one of the major dietary factors affecting the PPBG levels in DM ie, as well as in GDM.

The practical implications of *SIRT1* gene polymorphism case-control studies in GDM women have an important role, which can further be associated with prevention of screening and may be beneficial in treatment strategies. Future studies in different populations should be carried out with these two SNPs (rs4746720 and rs10823112) in *SIRT1* gene among the different ethnicities which can explore the functional and biological mechanisms present in *SIRT1* gene. If we succeed in addressing these issues, then we may acquire a better understanding between the role of genetics in GDM, as well as its impact on maternal and neonatal health issues.

The main impact of this study was considered to be the screening of *SIRT1* SNPs in the GDM women, and this study was considered to be the initial study carried out in GDM patients in Saudi Arabia. In this study, complete pregnant women (n = 240) were enrolled from Saudi ethnicity based on 75 g of OGTT tests. Recruiting all the patients from a single tertiary center will be considered as one of the limitations of this study. Missing out serum levels, restricting the screening with a couple of SNPs in *SIRT1* gene and regulating to 120 GDM cases and controls will be considered as the limitations of this study.

Conclusions

This study concludes that rs4746720 SNP was associated, and also with combination of clinical, biochemical and genotype data, confirming the glucose levels influence *SIRT1* gene SNPs. Future studies will be recommended to carry out these types of studies in the global population.

Data Sharing Statement

Based upon the reasonable interest, the corresponding author can share the data.

Ethical Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board in College of Medicine at King Saud University (E-22-7318; 10th January 2023-approved date). All the pregnant women participated in this study have confirmed and signed the written consent form before the participation in this study.

Consent for Publication

The pregnant women have provided the written informed consent form for this study.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no potential conflicts of interest with respect to the research, authorship, and/or publication of this research article.

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