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CLINICAL CORRELATION AND GENETIC EVALUATION OF SIRTUIN1 IN CORONARY ARTERY DISEASE AMONG SOUTH INDIAN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

Background and Objective: Studies evidenced that silent information regulator 1 (SIRT1), a NAD^+ dependent deacetylase has significant metabolic regulator, oxidative stress, and vascular function. It is involved in associations with coronary heart disease (CHD) risk and type 2 diabetes (T2D). Single-nucleotide polymorphisms (SNPs) of SIRT1 are associated with diabetes, blood pressure (BP), cholesterol metabolism and coronary artery calcification. Our study aimed to investigate the A>G SIRT1 SNPs, (rs3818291) in association of CHD with T2DM in South Indian patients.

Methods: A case-control study was performed on 225 subjects divided into healthy controls (n=75), T2DM without CAD (n=75), and T2DM with CAD (n=75). Biochemical parameters were estimated using automated analysers. Serum Sirtuin (SIRT1) and Sestrin 2 concentrations were determined by ELISA. Genotyping of the rs3818291 polymorphism for SNP was done by PCR. Statistical investigation was done by ANOVA, chi-square test, regression, and ROC curve analysis.

Results: SIRT1 levels were significantly maximum in T2DM patients with CAD than in other groups ($p<0.001$). Multivariate regression analysis revealed SIRT1 as an independent predictor of CAD (adjusted OR=2.21; $p=0.0019$). ROC curve analysis showed excellent discriminatory ability (AUC=0.864), with an optimal cut-point of 2.804 (82.3% sensitivity and 78.5% specificity). The rs3818291 polymorphism was significantly related with CAD risk in diabetic patients, with the GG genotype being a risk factor under the recessive model ($p<0.001$). Although Sestrin 2 levels were increased in CAD patients, it failed to maintain its independent predictive value.

Conclusion: SIRT1 and the polymorphism rs3818291 are significantly associated with CAD in South Indian patients with T2DM. These results specify that the combination of SIRT1 biomarker analysis and genetic profiling could improve the risk assessment of CAD in this high-risk group. Further studies needed to investigate to provide a novel therapeutic approach in patients with CAD.

KEYWORDS: Sirtuin 1; Polymorphism; Type 2 Diabetes Mellitus; Coronary Artery Disease; Sestrin 2

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) has developed a serious global public health issue, with over 537 million adults affected worldwide, and the prevalence is predictable to surge significantly in the succeeding few decades [1]. The South Asian region is characterized by high prevalence rates of T2DM, in spite of having a young population with relatively low levels of obesity. Cardiovascular complications, especially coronary artery disease (CAD), are responsible for the largest proportion of morbidity and mortality in people with T2DM [2]. Atherosclerotic cardiovascular disease remains the principal cause of death and disability among patients with diabetes mellitus, especially in those with T2DM in whom it typically occurs 14.6 years earlier [3].

The relationship between T2DM and CAD is complex, with chronic hyperglycemia-induced endothelial dysfunction, oxidative stress, the development of advanced glycosylation end-products, and chronic vascular inflammation all contributing to accelerated atherosclerosis [4]. There is ample clinical evidence that insulin resistance increases the risk for coronary artery disease (CAD) even in the absence of hyperglycemia [5]. Sirtuin 1 (SIRT1) is an evolutionarily conserved NAD^+ dependent deacetylase that regulates various components of cellular metabolism related to aging, DNA repair, mitochondrial biogenesis, and apoptosis [6]. The timely recognition of people with diabetes at increased risk of CVD is a major clinical imperative. SIRT1, a class III NAD^+-dependent histone deacetylase, acts as a metabolic and redox sensor, which plays a regulatory role in glucose metabolism, mitochondrial biogenesis, lipid metabolism, and endothelial nitric oxide synthase (eNOS) activity [7].

SIRT1 plays a crucial role in glucose-dependent insulin secretion, gluconeogenesis, regulating inflammation, lipolysis, and β-cell survival. Its association with various histones and nonhistone substrates has a significant impact on the development and advancement of diabetes, particularly T2DM [8]. Apart from the circulating protein concentration, genetic polymorphisms in the SIRT1 gene could affect transcriptional activity, enzyme function, or regulatory efficiency.

SIRT1 gene polymorphisms have been linked to metabolic phenotypes, obesity, and cardiovascular risk factor profiles in various populations, indicating the possible modulating effect on cardiometabolic susceptibility [9,10].
Sestrin 2, a stress-inducible protein that regulates oxidative stress, phosphorylates and inhibits mammalian target of rapamycin (mTOR) signaling, thus playing a role in maintaining redox balance in cells [11]. Circulating levels of Sestrin 2 have been revealed to be abnormal in cardiometabolic diseases, but its relationship with CAD in T2DM is not well understood [12]. Although SIRT1 has been explored in the context of metabolic dysfunction, there is a lack of evidence regarding the assessment of SIRT1 concentrations in circulation and SIRT1 gene polymorphisms in relation to CAD risk. Therefore, in our case control study aimed to investigate the SIRT1 gene polymorphism in T2DM with or without CAD.

MATERIALS AND METHODS

Study design and setting

This hospital-based case-control study was carried out at a tertiary care teaching hospital in South India and was approved by the Institutional Ethics Committee (IEC-SVMCHRI). The written informed consent was obtained from all participants before inclusion in the study.

Study population and group

A total of 225 participants were recruited and categorized into three groups:

- Group I- Healthy controls (n = 75)
- Group II- Patients with T2DM without CAD (n = 75)
- Group III- Patients with T2DM and confirmed CAD (n = 75)

T2DM was diagnosed based on the American Diabetes Association standards, including fasting plasma glucose ≥126 mg/dL. HbA1c ≥6.5%, or documented history of diabetes under treatment. CAD diagnosis was confirmed by clinical evaluation supported by electrocardiography, cardiac biomarkers, and coronary angiography signifying ≥50% luminal stenosis in at least one major coronary artery.

Inclusion and exclusion criteria

Inclusion criteria for diabetic groups included individuals aged ≥30 years with established T2DM. For Group III, angiographic confirmation of CAD was mandatory. Exclusion criteria included type 1 diabetes mellitus, chronic inflammatory disorders, malignancy, hepatic failure, chronic kidney disease stage IV or V, autoimmune diseases, recent acute infections, and use of antioxidant or immunomodulatory medications within three months prior to enrolment.

Clinical and biochemical evaluation

Demographic and clinical details, such as age, sex, and duration of diabetes, were obtained through a structured questionnaire. Venous blood samples were collected after an overnight fast of 8–10 hours. Biochemical parameters were measured using an automated clinical chemistry analyzer (Vitros Chemistry System 5.1 FS, Ortho Clinical Diagnostics, USA) according to manufacturer protocols. Glycosylated Hb (HbA1c) was measured using standardized high-performance liquid chromatography (HPLC). Creatine Kinase-MB (CK-MB) and Troponin I were quantified using chemiluminescent immunoassay techniques.

Measurement of serum Sirtuin 1 and Sestrin 2

Serum was separated by centrifugation at 3000 rpm for 10 min and stored at −80°C until analysis. Serum SIRT 1 and Sestrin 2 concentrations were quantified using commercially available ELISA kits according to the manufacturer's protocol.

Genotyping of SIRT1 rs3818291 polymorphism by PCR

Genomic DNA was extracted from peripheral venous blood using the Lupex DNA extraction kit (BDS-50). The purity and concentration were evaluated spectrophotometrically, and samples with an A260/A280 ratio between 1.8 and 2.0 were used for further analysis. Genotyping of the SIRT1 rs3818291 polymorphism was performed by PCR-based SNP genotyping method. Tetra-primer ARMS-PCR for genotyping the G/A SNP was performed in a 25 µL reaction containing 10–50 ng of genomic DNA, 1× PCR buffer with $MgCl_2$, 0.2 mM of each dNTP, and four primers adjusted to an initial inner and outer primer ratio of 2:1 (0.1 µM each of the outer primers spanning positions 365–392 and 672–645, and 0.2 µM each of the G- specific forward inner primer at 472–501 and A- specific reverse inner primer at 526–501), along with 0.5–1.0 U of Taq DNA polymerase, made up to volume with nuclease-free water. Amplification was carried out with an initial denaturation at 95 °C for 3–5 min followed by 30–35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 30–40 s, with a final extension at 72 °C for 5 min and hold at 4 °C. PCR products were carryout on a 2-3% agarose gel in 1× TBE to clearly separate the 162 bp, 202 bp, and 308 bp fragments; 5–10 µL of amplified product was loaded with tracking dye alongside a 100 bp DNA ladder. The assay generated a 202 bp allele-specific fragment for the G allele, a 162 bp fragment for the A allele, and a 308 bp outer–outer control fragment, that GG homozygotes showed bands at 308 bp and 202 bp only, AA homozygotes at 308 bp and 162 bp only, and AG heterozygotes at all three sizes (308 bp, 202 bp, and 162 bp), with known GG, AA, and AG controls (or sequenced references) included where available to validate genotyping accuracy.

Primer sequence

Forward inner primer (G allele): GAAGTGTTTAATAGTGCTCTGTATGTTTTG
Reverse inner primer (A allele): CTTATTTTTCCATCCCCTAGAGACAT
Forward outer primer (5' - 3'): AGGTTAATCGATAGGGTAGCTTTATGTA

Reverse outer primer (5' - 3'): TATTTAACCTCTATGGTCCTGAGTTTTC

Statistical analysis

Statistical analysis was carried out using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Continuous data were presented as mean ± SD and compared using one-way ANOVA with Tukey's post hoc test. Binary logistic regression analysis was performed to determine the independent predictors of CAD among diabetic patients, including age, HbA1c, LDL cholesterol, CK-MB, plasma SIRT 1, and Sestrin 2. Adjusted odds ratio (OR) with 95% confidence interval (CI) was calculated. Receiver operating characteristic (ROC) curve analysis was done to assess the diagnostic accuracy of circulating biomarkers. The area under the curve (AUC) and the best cut-off point were determined using the Youden index. Hardy-Weinberg equilibrium was tested for the SIRT1 gene rs3818291 polymorphism in the control population. Allele frequency was determined by direct gene counting. The association between rs3818291 genotypes and CAD risk in T2DM patients was analyzed using Chi-square test and OR with 95% CI for dominant (AG+GG vs. AA) and recessive (GG vs. AG+AA) models. A two-tailed p-value < 0.05 was considered statistically significant.

Results

Clinical and biochemical characteristics

A total of 225 participants were included in the study, consisting of 75 healthy controls, 75 patients with T2DM without CAD, and 75 patients with T2DM and angiographically proven CAD. The age distribution varied significantly among the three groups ($p < 0.001$), with the highest mean age recorded in the CAD group (59.827 ± 10.910 years), and followed by the T2DM without CAD group (53.440 ± 9.443 years) and the control group (47.853 ± 9.650 years). Fasting blood glucose (FBS), postprandial blood glucose (PPBS), and HbA1c levels showed highly significant differences among the groups ($p<0.001$ for all). Both diabetic groups showed significantly higher glycemic levels than the control groups. HbA1c levels were highest in T2DM without CAD (10.779 ± 1.988%) and remained high in T2DM with CAD (9.197 ± 2.240%), reflecting chronic hyperglycemia.

The changes in atherogenic lipids were more evident in diabetic patients, especially in those with CAD. Total cholesterol, triglycerides, LDL cholesterol, and VLDL were significantly different among the groups ($p<0.001$). The greatest increase was observed in LDL cholesterol in the CAD group (129.913 ± 30.099 mg/dL). HDL cholesterol did not show significant differences among the groups. The renal function markers showed significant differences among the groups. Urea and uric acid levels were significantly higher in the CAD group than in controls and T2DM without CAD ($p<0.001$). Serum creatinine levels were also significantly different ($p=0.043$), with higher mean values in the CAD group. The cardiac injury markers showed the most striking differences. CK-MB and Troponin I levels were significantly higher in T2DM patients with CAD than in controls and T2DM without CAD ($p<0.001$), indicating myocardial injury. Post-hoc Tukey test confirmed that patients with CAD had significantly higher levels of LDL cholesterol, CK-MB, troponin I, urea, uric acid, and age than controls and T2DM without CAD, with no significant differences between the two diabetic groups for FBS, total cholesterol, triglycerides, VLDL, and creatinine. A detailed summary of the baseline clinical and biochemical parameters is provided in Table 1.

Table 1. Baseline clinical and biochemical characteristics of study participants (ANOVA with Tukey Post Hoc)

Variable	Controls (n = 75)	T2DM (n = 75)	T2DM + CAD (n = 75)	p-value
Age (years)	47.85 ± 9.65[a]	53.44 ± 9.44[b]	59.83 ± 10.91[c]	<0.001
FBS (mg/dL)	96.07 ± 8.70[a]	160.76 ± 53.88[b]	161.33 ± 39.88[b]	<0.001
PPBS (mg/dL)	123.35 ± 13.60[a]	260.37 ± 70.14[c]	239.53 ± 59.46[b]	<0.001
HbA1c (%)	5.40 ± 0.30[a]	10.78 ± 1.99[c]	9.20 ± 2.24[b]	<0.001
Total Cholesterol (mg/dL)	172.96 ± 25.31[a]	199.56 ± 38.57[b]	209.30 ± 31.73[b]	<0.001
Triglycerides (mg/dL)	119.65 ± 27.87[a]	177.17 ± 56.24[b]	182.68 ± 66.58[b]	<0.001
LDL (mg/dL)	88.97 ± 14.60[a]	101.28 ± 27.35[b]	129.91 ± 30.10[c]	<0.001
VLDL (mg/dL)	28.79 ± 12.74[a]	36.48 ± 8.31[b]	35.08 ± 10.82[b]	<0.001
Urea (mg/dL)	21.43 ± 5.91[a]	22.92 ± 6.38[a]	27.13 ± 10.71[b]	<0.001
Uric Acid (mg/dL)	4.90 ± 0.71[a]	4.74 ± 0.95[a]	5.96 ± 1.11[b]	<0.001
CK-MB (U/L)	17.85 ± 7.05[a]	17.71 ± 6.63[a]	42.27 ± 29.34[b]	<0.001
Troponin I (ng/mL)	0.019 ± 0.016[a]	0.039 ± 0.047[a]	0.672 ± 1.102[b]	<0.001

Values are expressed as mean ± SD. Intergroup comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. Different superscript letters (a, b, c) indicate statistically significant differences between groups ($p < 0.05$)

Sirtuin 1 and Sestrin 2 levels

Serum levels of SIRT 1 showed a highly significant difference between the three study groups ($p<0.001$). SIRT 1 levels were significantly higher in patients with T2DM and angiographically proven CAD (7.286 ± 6.450) than in those with T2DM but without CAD (2.072 ± 1.490) and in healthy controls (1.238 ± 1.081). There was a definite stepwise rise from healthy controls to patients with T2DM and then to patients with T2DM and CAD, reflecting progressive SIRT 1

dysregulation with increasing cardio metabolic risk. The extent of intergroup variability was strong and reflected high discriminatory power. The levels of SIRT 1 in the three study groups are depicted in boxplot Figure 1 and Table 2. The levels of Serum Sestrin 2 were also significantly different among the groups ($p<0.001$). The mean levels of Sestrin 2 were lowest in the control group (0.205 ± 0.143), slightly higher in T2DM without CAD (0.216 ± 0.267), and much higher in T2DM with CAD (1.348 ± 2.818). Although the values were higher in the CAD group, the variability in this group was found to be higher compared to SIRT 1, indicating lower discriminative stability. The boxplot distribution profile of Sestrin 2 is represented in Figure 2 and Table 2.

Figure 1 – Boxplot of serum Sirtuin 1 across groups

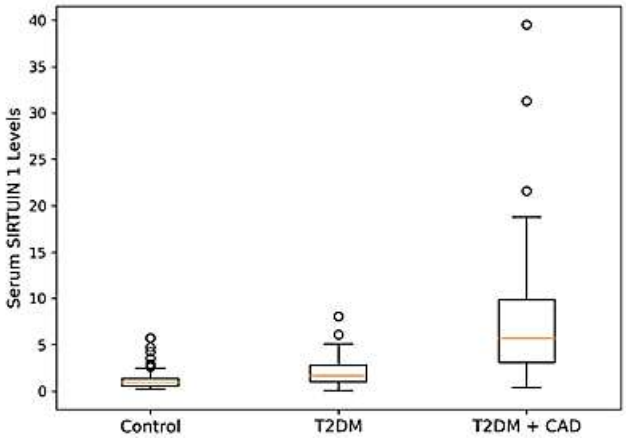


Figure 2 – Boxplot of Serum Sestrin 2 across groups

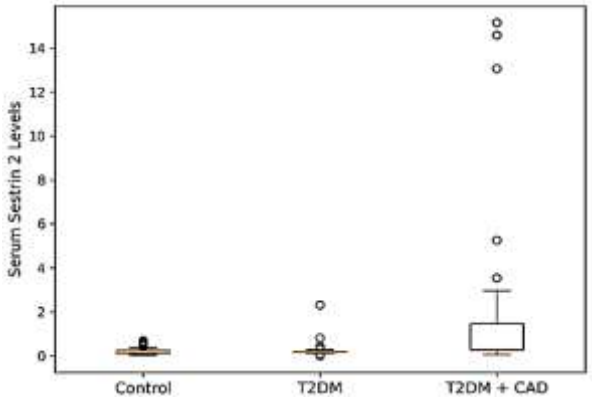


Table 2. Circulating molecular biomarkers

Biomarker	Controls (n = 75)	T2DM (n = 75)	T2DM + CAD (n = 75)	p-value
SIRTUIN 1	1.238 ± 1.081[a]	2.072 ± 1.490[b]	7.286 ± 6.450[c]	<0.001
Sestrin 2	0.205 ± 0.143[a]	0.216 ± 0.267[a]	1.348 ± 2.818[b]	<0.001

Values are expressed as mean ± SD. Intergroup comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. Different superscript letters (a, b, c) indicate statistically significant differences between groups ($p < 0.05$).

Correlation and independent predictive analysis

Pearson correlation analysis was carried out to study the relationship between the biochemical and molecular parameters in the diabetic population (n = 150). High positive correlations were found between HbA1c and fasting blood glucose ($r = 0.604$, $p < 0.001$) and postprandial blood glucose ($r = 0.651$, $p < 0.001$), which indicates the combined process of short- and long-term glucose control in T2DM.

Serum SIRT 1 levels showed a moderate positive correlation with LDL cholesterol ($r = 0.347$, $p < 0.001$) and a stronger correlation with CK-MB ($r = 0.512$, $p < 0.001$), suggesting its relationship with atherogenic lipid profiles and myocardial injury. A moderate positive correlation was also found between SIRT 1 and Sestrin 2 ($r = 0.444$, $p < 0.001$), suggesting a degree of convergence in stress signaling pathways. Sestrin 2 also showed a moderate correlation with CK-MB ($r = 0.403$, $p < 0.001$), although the magnitude of association was weaker than that of SIRT 1 with CK-MB.

To determine the independent predictors of CAD in T2DM patients (n = 150), multivariate binary logistic regression analysis was performed, including age, HbA1c, LDL cholesterol, CK-MB, SIRT 1, and Sestrin 2. In the adjusted model, SIRT 1 remained an independent predictor of CAD (adjusted OR = 2.21; 95% CI: 1.34-3.65; $p = 0.0019$), suggesting that elevated levels significantly increased the odds of CAD. LDL cholesterol ($p = 0.028$) and CK-MB ($p = 0.009$) were also independent predictors in the adjusted model. Age was borderline ($p = 0.058$). HbA1c was inversely associated with CAD

in the adjusted model, which is likely due to interaction or collinearity with other metabolic covariates. Sestrin 2 was no longer an independent predictor after adjustment for other variables. The full regression model is shown in Table 3.

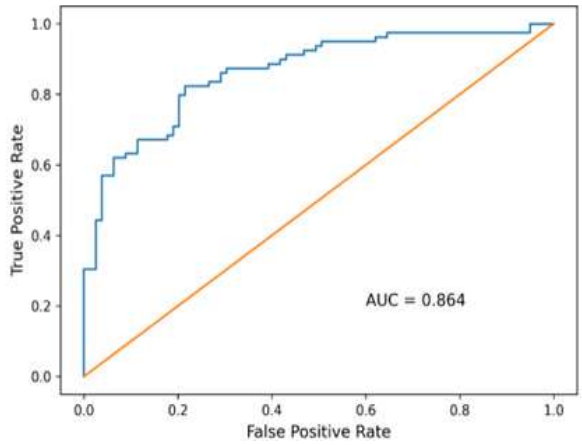
Table 3. Multivariate Logistic Regression analysis for CAD among T2DM Patients (n = 150)

Variable	Adjusted OR	95% CI	p-value
Age	1.09	0.99 – 1.19	0.058
HbA1c	0.25	0.13 – 0.49	<0.001
LDL	1.03	1.00 – 1.07	0.028
CK-MB	1.12	1.03 – 1.21	0.009
SIRTUIN 1	2.21	1.34 – 3.65	0.0019
Sestrin 2	3.08	0.55 – 12.94	0.241

Diagnostic performance

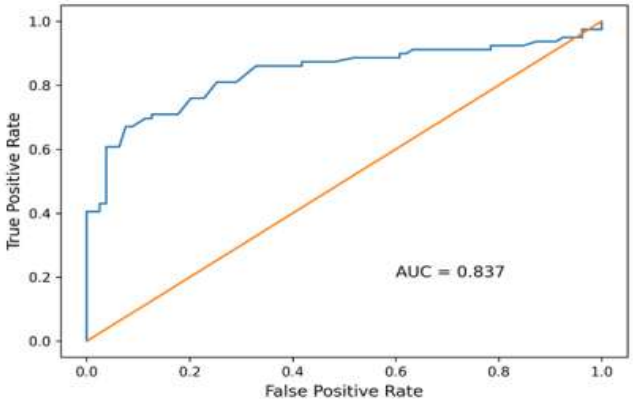
Analysis of the ROC curve was conducted to assess the discriminative power of circulating biomarkers in distinguishing T2DM patients with CAD from those without CAD. Serum SIRT 1 showed excellent diagnostic performance with an area under the curve (AUC) of 0.864 (Figure 3), indicating high discriminative power.

Figure 3 – ROC Curve for SIRTUIN 1



With the optimal cut-off point of 2.804, as determined by the Youden index, SIRT 1 showed a high sensitivity of 82.3% and specificity of 78.5%, indicating a good balance in diagnostic performance. CK-MB also showed high discriminative power with an AUC of 0.837 (Figure 4).

Figure 4 – ROC Curve for CK-MB



With the optimal cut-off point of 27.0 U/L, CK-MB showed a higher specificity of 92.4% but a relatively lower sensitivity of 67.1% compared to SIRT 1. Although CK-MB showed high confirmatory value because of its high specificity, it showed relatively lower overall diagnostic performance compared to SIRT 1. These results indicate that circulating SIRT

1 has comparable diagnostic performance to existing cardiac biomarkers and may be used as a useful adjunct biomarker for the diagnosis of CAD in T2DM patients.

rs3818291 genotype distribution and association with CAD risk

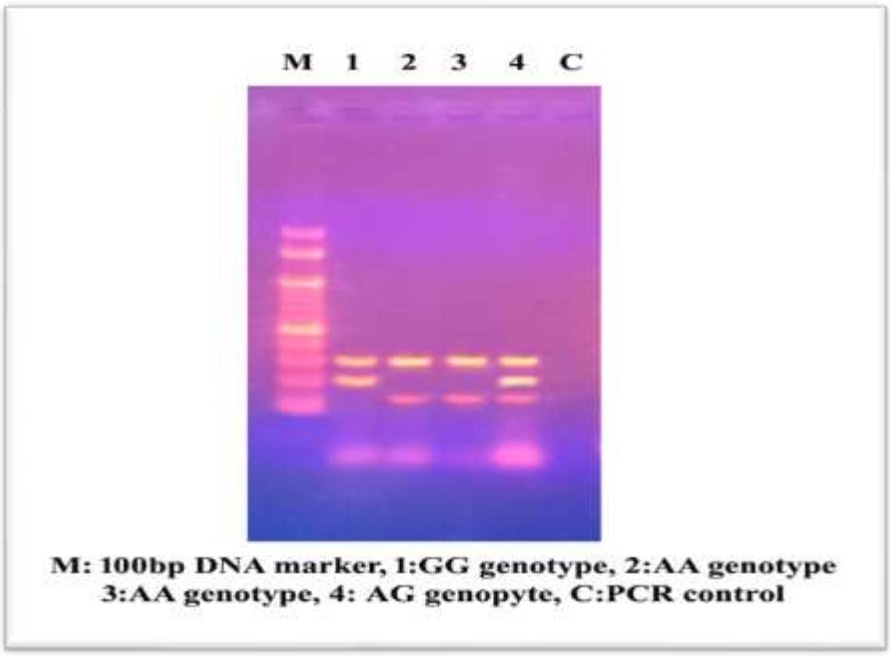
The distribution of the genotype of SIRT1 rs3818291 was significantly different among the study groups (Table 4 & Figure 5). Although the AA genotype was most prevalent in controls (97.3%) and remained so in T2DM patients without CAD (68.0%), there was a significant change in T2DM patients with CAD, where the GG genotype accounted for 48.0%. This was reflected in the frequency of the G allele, which rose from 22.0% in T2DM patients without CAD to 56.7% in those with CAD. In diabetic patients, the most prevalent genetic model (AG+GG vs AA) showed a significantly increased odds of CAD by nearly four times (OR = 3.99; 95% CI: 2.05–7.77; $p < 0.001$). Using the recessive model (GG vs AG+AA), the odds of CAD were 6.77 times higher in individuals with the GG genotype (95% CI: 2.90–15.80; $p < 0.001$). The results clearly show a strong and significant association between the rs3818291 polymorphism and CAD risk in T2DM patients.

Table 4. Genotype and allele distribution of SIRT1 rs3818291 among study groups

Genotype / Model	Controls (n=75)	T2DM (n=75)	T2DM + CAD (n=75)	OR (95% CI)*	p-value
AA	73 (97.3%)	51 (68.0%)	26 (34.7%)	—	—
AG	2 (2.7%)	15 (20.0%)	13 (17.3%)	—	—
GG	0 (0.0%)	9 (12.0%)	36 (48.0%)	—	—
G allele frequency	0.013	0.220	0.567	—	—
Dominant model (AG+GG vs AA)†	—	24 vs 51	49 vs 26	3.99 (2.05–7.77)	<0.001
Recessive model (GG vs AG+AA)†	—	9 vs 66	36 vs 39	6.77 (2.90–15.80)	<0.001

**Odds ratios calculated for T2DM + CAD versus T2DM without CAD. Genetic model comparison performed among diabetic patients only. Genotype distribution in controls conformed to Hardy–Weinberg equilibrium (p > 0.05).*

Figure 5. Agarose gel image depicting PCR band patterns for SIRT1 rs3818291 genotypes (AA, AG, GG)



DISCUSSION

Clinical and cardiometabolic profile of the study population

The current study showed that there were marked differences in the age distribution among the study groups, with increased mean age in T2DM patients with CAD. This is consistent with the well-recognized concept that increasing age is a principal non-modifiable risk factor for CAD, especially in the presence of underlying chronic metabolic disturbances [13]. The cumulative effects of aging on endothelial dysfunction, oxidative stress, and vascular injury could play a synergistic role in the concomitant presence of CAD among older diabetic patients. There were large differences among the study groups in glycemic indices, with increased fasting and postprandial glucose concentrations in diabetic patients. HbA1c showed large variability among the study groups, indicating underlying chronic glycemic exposure. Chronic hyperglycemia is known to accelerate vascular injury due to the formation of advanced glycosylation end-products, protein kinase C activation, and increased oxidative stress, thereby contributing to accelerated atherosclerosis [4]. The

large increase in HbA1c concentrations among diabetic patients emphasizes its use as a marker of cumulative metabolic risk.

Lipid variables further emphasized the presence of an atherogenic profile in T2DM patients with CAD, especially the high levels of LDL cholesterol. LDL cholesterol is a major contributor to atheroma development due to oxidation and macrophage-mediated uptake, leading to the instability of atherosclerotic plaques and inflammation of the vascular wall [14]. The lack of significant differences in HDL cholesterol levels among the groups indicates that the measurement of HDL cholesterol levels may not adequately reflect the functional deficits of diabetic dyslipidemia, where qualitative changes are more predominant. The cardiac biomarkers showed the greatest degree of increase in the CAD group, with significantly higher CK-MB and Troponin I levels. Troponin I, a highly specific marker of myocardial damage, indicates cardiomyocyte death even in the absence of clinical ischemic events [15]. The increased levels found in diabetic patients with CAD are consistent with the presence of chronic or previous myocardial damage and validate the clinical grouping of the CAD group. Taken together, these data provide evidence of a unique cardio metabolic risk factor profile in T2DM patients with CAD, marked by the presence of advanced age, chronic hyperglycemia, atherogenic dyslipidemia, and biochemical evidence of myocardial injury.

SIRTUIN 1 and Sestrin 2 in cardiometabolic dysfunction

The current study showed a significant and progressive increase in the level of circulating SIRT 1 from healthy controls to T2DM patients and then to those with established CAD. This progressive increase in SIRT 1 levels could indicate that SIRT 1 is a potential biomarker of increasing cardio metabolic stress rather than just glycemic stress. SIRT 1 is known to be a NAD^+-dependent deacetylase that regulates endothelial nitric oxide synthase activity, mitochondrial biogenesis, and inflammatory signaling pathways [16]. There is experimental evidence to suggest that SIRT 1 regulates vascular homeostasis through the deacetylation of key transcription factors that regulate oxidative stress and endothelial cell survival [17]. The current study's findings of increased circulating levels of SIRT 1 may therefore indicate a compensatory mechanism for chronic oxidative and metabolic stress in diabetic patients with vascular injury. Previous translational research has indicated that there is a close association between the dysregulation of SIRT 1 and endothelial dysfunction and the rapid progression of atherosclerosis, especially under hyperglycemic conditions [18]. Under diabetic conditions, the metabolic overload may lead to the adaptive upregulation of stress response pathways, such as the SIRT pathway, as a compensatory mechanism against vascular inflammation and instability [19]. The incremental increase observed in the present study is consistent with this understanding and places SIRT 1 in the bloodstream as a marker of cumulative cardiometabolic risk.

Serum Sestrin 2 levels were also found to be significantly increased in T2DM patients with CAD; however, the higher variability noted in the CAD group indicates relatively lower discriminatory stability. Sestrin 2 has been shown to activate AMP-activated protein kinase (AMPK) and suppress mTOR signaling in response to oxidative stress [20]. The increase noted in Sestrin 2 levels in CAD may thus indicate the activation of cellular stress response pathways rather than a direct role in the atherogenic process. Clinical studies have noted an increase in Sestrin 2 levels in CVD, which may potentially indicate an endogenous protective response to ischemic injury [21]. The variability noted in the CAD group in the current study may thus indicate differences in the activation of stress response pathways among individuals with differing levels of myocardial injury. These findings collectively indicate that while both SIRT 1 and Sestrin 2 are stress-responsive, SIRT 1 has higher discriminatory power and may potentially better correlate with the severity of vascular involvement in T2DM patients.

Correlation and independent predictive role of SIRTUIN 1

The strong inter-relationships among the glycemic indices in the current study are indicative of the integrated processes of short- and long-term glucose control in T2DM. Hyperglycemia is known to cause chronic endothelial stress, inflammation, and vascular remodeling, thus potently increasing cardiovascular risk [22]. For instance, the downregulation of SIRT1 by hyperglycemia caused vascular dysfunction in DM [23]. The moderate correlations between SIRT 1 and both LDL cholesterol and CK-MB indicate that plasma SIRT 1 concentration may be affected by converging metabolic and ischemic stress pathways rather than by individual glycemic disturbances. It is important to note that SIRT 1 was independently associated with CAD after multivariate adjustment for age, HbA1c, LDL cholesterol, CK-MB, and Sestrin 2. This indicates that SIRT 1 may reflect a dimension of cardiovascular risk that is not adequately accounted for by established metabolic and lipid risk factors. SIRT 1 has been demonstrated to modulate endothelial nitric oxide availability, inhibit vascular smooth muscle cell proliferation, and suppress inflammatory transcriptional activity [24]. These processes have been directly linked to the pathogenesis of plaque instability and atherothrombosis, thus providing a biological rationale for the independent association between SIRT 1 and CAD.

Conversely, Sestrin 2 did not retain independent predictive value after adjustment for covariates. While Sestrin 2 is known to be induced by oxidative stress and metabolic dysregulation, its plasma concentration could represent a more general phenomenon of stress adaptation rather than atherogenic engagement per se [25]. The reduction in significance with multivariate modeling may imply that its relationship with CAD could be mediated by shared pathways with lipid dysregulation or myocardial injury. The retention of LDL cholesterol and CK-MB as independent predictors is in keeping with their recognized roles in atherosclerotic burden and myocardial injury, respectively. Notably, the independent predictive value of SIRT 1 suggests that it could offer additional risk information beyond conventional cardiometabolic risk factors.

Diagnostic performance of SIRTUIN 1

ROC analysis showed that circulating SIRT 1 has high discriminative power for the detection of CAD in T2DM patients, with an AUC of 0.864. A value of AUC > 0.80 is generally regarded as a criterion for excellent diagnostic accuracy in clinical biomarker studies [26]. The good sensitivity (82.3%) and specificity (78.5%) at the optimal cut-off point of 2.804 also indicate that SIRT 1 could be useful in the risk stratification of CVD rather than being a confirmatory test. More importantly, the diagnostic accuracy of SIRT 1 was not significantly different from that of CK-MB (AUC = 0.837), an established biomarker of myocardial damage. Although CK-MB had higher specificity, SIRT 1 had higher sensitivity and a more balanced ratio of false positives to false negatives at the optimal cut-off point. This difference could be important in the early detection of high-risk diabetic patients, who need to be identified before the onset of overt myocardial necrosis due to chronic vascular stress. Unlike CK-MB, which is a marker of acute cardiomyocyte injury, SIRT 1 may reflect upstream metabolic and endothelial derangements that are associated with the pathogenesis of atherosclerosis [27]. Recent findings indicate that biomarkers of vascular dysfunction and oxidative stress could improve the prediction of cardiovascular risk beyond the traditional markers of myocardial injury [28]. The high AUC value found in the current study supports the hypothesis that circulating SIRT 1 could be a biomarker that combines information from metabolic dysregulation, endothelial stress, and inflammation. While these results are promising for their diagnostic potential, it is likely that the performance of a biomarker can differ in various populations. Validation studies are therefore needed to establish whether SIRT 1 can improve the accuracy of prediction beyond current models of cardiovascular risk.

Association of rs3818291 with CAD risk in T2DM

The current study has shown a strong and statistically significant link between the SIRT1 rs3818291 polymorphism and CAD risk in patients with T2DM. The significant overrepresentation of the GG genotype in the CAD group, as well as the significantly increased odds under both dominant and recessive models, indicate that the G allele could potentially be involved in the increased susceptibility to coronary complications in the studied diabetic population. SIRT1 is a key regulator of vascular homeostasis, modulating endothelial nitric oxide synthase activity, mitochondrial function, inflammatory responses, and cellular stress responses [29]. SIRT1 downregulation has been associated with endothelial dysfunction, increased oxidative stress, and accelerated atherogenesis (Stein and Matter, 2011). Although the rs3818291 polymorphism is located in an intronic region, intronic variants can potentially affect gene expression by modulating transcriptional regulation, mRNA stability, or linkage disequilibrium with functional regions [30]. The strong association found in the current study indicates that this variant, or a variant in linkage disequilibrium with a functional polymorphism, could potentially affect SIRT1-mediated vascular protective functions in patients with chronic hyperglycemia. Diabetes is known to be associated with chronic oxidative stress, accumulation of advanced glycosylation end-products, and low-grade inflammation, all of which contribute to the acceleration of atherosclerosis [31].

Genetic variants that impair protective signaling pathways, such as SIRT1, may therefore enhance the risk of vascular injury in this high-risk metabolic milieu. The significantly high odds ratio under the recessive model suggest that homozygous carriers of the G allele may be at a highly increased risk of vulnerability to coronary pathology. Although previous studies have investigated the association of SIRT1 polymorphisms with metabolic disorders, the results have been inconsistent across different populations, possibly due to the heterogeneity of ethnic groups and gene-environment interactions [9]. The current study offers region-specific data from the South Indian population, focusing on rs3818291 as a potential genetic risk factor for CAD in patients with T2DM. Collectively, these results support a contributory role for rs3818291 in the susceptibility to coronary disease in diabetic patients, potentially mediated by the modulation of SIRT1-dependent vascular protective mechanisms.

CONCLUSION

This study shows that SIRT 1 concentrations are significantly increased in South Indian patients with T2DM and angiographically proven CAD. SIRT 1 was identified as an independent predictor of CAD in a multivariate model and had excellent diagnostic performance with high discriminative power comparable to that of established cardiac biomarkers. These data suggest that SIRT 1 concentrations may have a role as a clinically useful cardiometabolic biomarker for risk stratification in diabetic patients. Moreover, the SIRT1 rs3818291 polymorphism had a strong and statistically significant association with the risk of CAD in T2DM patients, particularly in the recessive genetic model. The strong enrichment of the GG genotype in the CAD group indicates that genetic variation in SIRT1 may contribute to an increased susceptibility to coronary complications in this high-risk metabolic environment. These data collectively support a contributory role for SIRT1 in the pathophysiology of diabetic CAD. These data point to the potential value of combining circulating SIRT 1 measurement and genetic analysis to improve cardiovascular risk assessment.

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