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Original article

Study of the Relationship Between Sirtuin 1 Rs7069102 Polymorphism and Diabetic Nephropathy in Egyptian Patients with Type 2 Diabetes Mellitus

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Abstract

Background: Type 2 diabetes mellitus (T2DM) is characterized by chronic hyperglycaemia resulting from defects of insulin action, insulin secretion or both, causing macrovascular and microvascular complications, which can affect kidneys causing diabetic nephropathy (DN) which is one of the most feared diabetic chronic microvascular complications. Sirtuin 1 (SIRT1) is a NAD+-dependent deacetylase, belongs to a group of enzymes called silent information regulators. The aim of this work was to study the association between Sirtuin 1 rs7069102 polymorphism and diabetic nephropathy in Egyptian patients with T2DM. **Methods:** This cross-sectional study was carried on 85 patients, who had T2DM for at least 10 years for assessment of the relationship between SIRT1 rs7069102 polymorphism and DN in patients with T2DM. Patients were subdivided into two groups: group I: included (45 patients) with T2DM and DN, and group II: included (40 patients) without DN. We analyzed the rs 7069102 polymorphism using StepOne real-time polymerase chain reaction (PCR) System and TaqMan SNP Genotyping assay. **Results:** The CC genotype showed a higher percentage in patients with DN compared to those without DN (42.2 vs 7.5%). The percentage of CG genotype was significantly higher in patients without DN compared to those with DN (52.5% vs 46.7%, p=0.005), and the GG genotype was also significantly more prevalent in patients without DN (40.0% vs 11.1%, p<0.001). **Conclusions:** The study highlighted the significant association between the SIRT1 rs7069102 polymorphism and the risk of diabetic nephropathy in patients with T2DM. SIRT1 rs7069102 was found to be an independent predictor of DN.

Keywords: Sirtuin 1 rs7069102, Polymorphism, Diabetic Nephropathy, Type 2 Diabetes Mellitus

Introduction:

The prevalence of diabetes mellitus is increasing globally. According to the latest global diabetes distribution map released by the International Diabetes Federation (IDF) in 2019, there were 463 million diabetic patients worldwide, with an increase of 11.57% in the global prevalence compared to 2015 (1).

Most of this increase comes from low- and middle-income countries, and the majority of these patients suffer from type 2 diabetes mellitus (T2DM). T2DM is a pathology of heterogeneous etiology characterized by chronic hyperglycaemia resulting from defects of insulin action, insulin secretion or both (2).

Hyperglycaemia causes macrovascular and microvascular complications, which can also affect kidneys. This condition is called diabetic nephropathy (DN) which is one of the most feared diabetic chronic microvascular complications and the leading cause of end-stage renal disease worldwide, which is associated with high morbidity and mortality. It affects 20-40 % of diabetic patients. DN is defined by persistent albuminuria (or albuminuria excretion rate of 300 mg/day or 200ug/min) and progressive decrease in glomerular filtration rate (GFR) which often occur in association with elevation in blood pressure, ultimately leading to end-stage renal disease (3).

Several association studies have identified a strong genetic component to both albuminuria and impaired glomerular filtration rate. Identifying genes that are involved in DN could lead to novel forms of treatment and may be even prevention of this life-threatening condition (4).

Sirtuin 1 (SIRT1) is a NAD+-dependent deacetylase, belongs to a group of enzymes called silent information regulators that are expressed in a variety of tissues including kidney, adipose tissue, muscle, liver, and pancreas. It modifies proteins that participate in DNA repair, stress, inflammatory response, and regulation of energy metabolism. SIRT1 promotes lipolysis in white adipose tissue, protects from excessive lipid accumulation in skeletal muscle and liver, and supports insulin secretion (5).

Sirtuin 1 (SIRT1) secretion is induced during periods of calorie restriction and indirectly inhibits stress-induced apoptosis. High insulin resistance could reduce the SIRT1 expression, and then decrease the insulin sensitivity. The human SIRT1 gene is located on chromosome 10q21.3, it contains 9 exons and 8 introns and encodes a protein composed of 747 amino acids residues (6).

The role of inflammation in diabetes related complications is very well established. SIRT1 suppresses inflammatory pathways in the cell, the expression of pro-inflammatory genes and the secretion of TNF-α, IL-1β, IL-6, p53, FoxO, and other pro-inflammatory molecules (7).

A study has specifically linked SIRT1 and chronic renal injury caused by inflammation. SIRT1 also has renoprotective effects, as it offers resistance to inflammation, apoptosis of tubular and glomerular cells and reduces interstitial fibrosis (8).

A number of studies showed that SIRT1 and its polymorphisms are involved in visceral obesity, which can lead to T2DM (9).

In this study, we investigated the relationship between the SIRT1 rs7069102 polymorphism and DN in patients with T2DM.

The aim of this work was to study the association between SIRT1 rs7069102 polymorphism and DN in Egyptian patients with T2DM.

Patients and Methods:

This cross-sectional study was carried out on 85 patients, who had T2DM for at least 10 years for assessment of the relationship between SIRT1 rs7069102 polymorphism and DN in patients with T2DM. The diagnosis of T2DM and DN was made according to the World Health Organization criteria. This study was performed in the Internal Medicine Department Benha University Hospital between March 2023 to September 2023.

An informed written consent was obtained from all patients. The study was done after approval from the Research Ethics Committee of Benha, Faculty of Medicine (approval code:MS:13-10-2022).

Exclusion criteria were overt nephropathy, active infection, poor glycaemic control (glycated haemoglobin HbA1C above 10), significant heart failure, alcoholism, and presence of other possible causes of renal disease.

Patients were divided into two groups; group I: included (45 patients) with T2DM and DN, and group II: included (40 patients) without DN.

All patients were subjected to detailed history taking, full clinical examination, diagnosis of diabetes based on plasma glucose criteria or HbA1C criteria.

Diagnosis of diabetic nephropathy (based on current guidelines using four main criteria: a decline in renal function, diabetic retinopathy, proteinuria, and a reduction in GFR. However, the hallmark of established DN is persistent albuminuria, with coexisting retinopathy and no evidence of alternative kidney disease.), laboratory investigation [Complete blood count, Lipid profile, kidney function test (urea and creatinine), glycated haemoglobin (HbA1C), estimated glomerular filtration rate, albumin creatinine ratio, and detection of Sirtuin 1 rs7069102 polymorphism].

Blood sampling: Six mls of venous blood were taken from each patient under complete aseptic conditions after overnight fasting. The sample was divided as follows: One ml whole blood was added to an EDTA-contained sterile tube and stored at -80 for subsequent genomic DNA extraction. Two ml of blood was added to another EDTA-contained sterile tube for CBC and Glycated Hb. Three ml blood was put in serum separating tubes and allowed to coagulate at room temperature then centrifuged at 3000 rpm for 10 minutes for serum separation. It was used for kidney function tests and lipid profile.

Urine sampling: Urine samples were obtained by mid-stream clean catching early in the morning for assessment of urine albumin and creatinine ratio.

Molecular detection of Sirtuin 1 single nucleotide polymorphism (SNP) (rs7069102) by real time polymerase chain reaction (RT-PCR) system and TaqMan SNP Genotyping Assay

All patients were genotyped for Sirtuin 1 rs7069102 SNP by using real time PCR analysis by Taqman probe assay in the following steps:

- Genomic DNA extraction from leucocytes.
- The extracted DNA was amplified using primers designed to detect the target polymorphism.
- Detection of PCR products by monitoring the increase of

fluorescence of a-dye labeled DNA probe.

Genomic DNA extraction:

Gene JET Whole Blood Genomic DNA Purification Mini Kit was used according to the instruction of the manufacturer for DNA purification (Thermo Scientific #K0781).

Amplification by Real-Time PCR:

Genotyping of Sirtuin 1 gene SNP (rs7069102) was done using the TaqMan SNP genotyping assays (LOT.No.01293521, Applied Biosystems, Thermo Fisher Scientific). The PCR was done using Veriti Real Time PCR instrument (S/N 2990226743, Applied Biosystems, Singapore) according to the instructions of the manufacturers. The volume of the reaction mix was 5ml. The reaction mix was comprised of TaqMan Universal Master Mix, oligonucleotide primers labelled with VIC/FAM fluorescent dyes, 1.88 ml of purified H2O, and 0.5ul of DNA. The following protocol was used for the amplification of DNA: Step 1, 30 second read of starting fluorescence at 60 C (pre PCR read)

Statistical analysis

The collected data was revised, coded, tabulated, and introduced to a PC using Statistical package for Social Science (SPSS 25) (IBM Inc., Chicago, IL, USA). Shapiro-Wilk test was done to test the normality of data distribution. Quantitative variables were presented as Mean, Standard deviation (± SD) and range for parametric data, while Median and Interquartile range (IQR) for non-parametric data. Frequency and percentage were used to present non-numerical data. Student T Test was used to assess the statistical significance of the difference between two study group means. Chi-Square test was used to examine the relationship between two qualitative variables. Fisher's exact test was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells. Correlation analysis (using Spearman's rho method) to assess the strength of association between two quantitative variables. The correlation coefficient denoted symbolically "r" defines the strength (magnitude) and direction (positive or negative) of the linear relationship between two variables. A two tailed P value < 0.05 was considered significant.

Results:

The subjects were selected randomly from population in Qalyubia Governorate in Egypt. They were unrelated. Applying Hardy Weinberg equation, calculated the expected count, and revealed that all studied SNP genotypes in control group, as well as in cases' groups were in HW equilibrium (i.e. no significant differences were found between observed and expected counts in each group. **(Table 1).**

There was no significant difference between the two groups regarding TLC, platelets, and haemoglobin. The difference regarding HbA1c, creatinine, urea, UACR, TG, and LDL levels was highly significant ($p<0.001$), indicating that individuals with DN had significantly higher HbA1c, creatinine, urea, UACR, TG, and LDL levels compared to those without DN. Regarding HDL levels, DN group had significantly lower HDL when compared to those without DN ($p=0.001$). For cholesterol levels, the DN group had a mean of 192.9 (±34.96) mg/dL, while the without DN group had a mean of 190.4

(±33.14) mg/dL, the difference was not significant (p=0.740). **(Table 2)**

The CC genotype showed a higher percentage in patients with DN compared to those without DN (42.2 vs 7.5%). The percentage of CG genotype was significantly higher in patients without DN compared to those with DN (52.5% vs 46.7%, p=0.005), and the GG genotype was also significantly more prevalent in patients without DN (40.0% vs 11.1%, p<0.001). The dominant model analysis showed that the combined CG+GG genotypes were significantly associated without DN (92.5% vs. 57.8%, p<0.001). Similarly, in the recessive model analysis, the GG genotype was significantly associated without DN (40% vs. 11.1%, p=0.002). The G allele frequency was significantly higher in patients without DN compared to those with DN (66.25% vs. 34.4%, p<0.001). For CG, GG genotypes, dominant, recessive model and G allele, had OR less than 1, which indicates that presence of CG, GG genotypes, dominant, recessive model and G allele had protective effects against DN occurrence. **(Table 3)**

There were no significant differences in genotype frequencies based on sex (p=0.911) or age (p=0.956). There were no significant differences in HbA1c levels, TLC, platelet count, haemoglobin levels, creatinine or urea levels, UACR, cholesterol, TG, HDL, or LDL levels among the different genotypes, suggesting that SIRT1 rs7069102 gene polymorphism may not have a significant impact on HbA1c, TLC, platelet count, haemoglobin levels, creatinine or urea levels, UACR, cholesterol, TG, HDL, or LDL levels in patients with DN. **(Table 4)**

Higher HbA1C, TG, LDL, lower HDL, absent CC or GG were associated with DN in univariable analysis. However, in multivariable analysis, only higher HbA1C, TG, LDL, absent CC or GG were considered independent predictors of DN occurrence. **(Table 5)**

Table 1: Assessment of Hardy Weinberg equilibrium for Genetic polymorphisms

		DN n = 45		Without DN n = 40	
		Observed	**Expected**	**Observed**	**Expected**
SIRT1 rs7069102	**CG**	19	19.34	3	4.56
	GC	21	20.32	21	17.89
	GG	5	5.34	16	17.56
	p	**0.823**		**0.271**	

Table 2: Comparison between DN and without DN regarding personal history, HbA1c, CBC, renal function tests, UACR, and lipid profile

		DN n = 45		Without DN n = 40		Test	p
		No.	**%**	**No.**	**%**		
Sex	**Male**	18	40.0	15	37.5	X^2=	0.813
	Female	27	60.0	25	62.5	0.056	
Age (years)	**Mean ± SD.**	65.31 ± 6.62		62.43 ± 9.71		t=	0.118
	Median	66.0		63.0		1.582	
	Min. – Max.	53.0 – 78.0		48.0 – 80.0			
HbA1c %	**Mean ± SD.**	7.86 ± 0.67		6.94 ± 0.35		U=	<0.001*
	Median	7.80		6.90		198.0*	
	Min. – Max.	6.90 – 9.0		6.50 – 8.0			
TLC (x10^9/L)	**Mean ± SD.**	7.39 ± 2.24		7.31 ± 2.50		t=	0.878
	Median	7.0		7.10		0.154	
	Min. – Max.	3.50 – 13.0		3.50 – 12.50			
Platelet	**Mean ± SD.**	208.9 ± 64.91		191.7 ± 79.06		t=	0.273
(x10^9/L)	**Median**	200.0		179.0		1.103	
	Min. – Max.	90.0 – 350.0		76.0 – 400.0			
Hb (g/dL)	**Mean ± SD.**	12.06 ± 1.57		12.35 ± 1.65		U=	0.406
	Median	12.0		12.0		993.5	
	Min. – Max.	10.0 – 15.0		10.0 – 16.0			
Creatinine	**Mean ± SD.**	4.13 ± 1.15		0.83 ± 0.17		t=	<0.001*
(mg/dL)	**Median**	4.0		0.80		19.142*	
	Min. – Max.	2.0 – 8.0		0.60 – 1.10			
Urea (mg/dL)	**Mean ± SD.**	125.6 ± 27.98		28.68 ± 5.76		t=	<0.001*
	Median	124.0		29.0		22.704*	
	Min. – Max.	70.0 – 185.0		20.0 – 40.0			
UACR (mg/g)	**Mean ± SD.**	653.6 ± 176.1		42.38 ± 21.77		U=	<0.001*
	Median	700.0		32.0		0.0*	
	Min. – Max.	310.0 – 898.0		19.0 – 120.0			
Cholesterol	**Mean ± SD.**	192.9 ± 34.96		190.4 ± 33.14		t=	0.740
(mg/dL)	**Median**	186.0		190.5		0.333	
	Min. – Max.	119.0 – 290.0		137.0 – 300.0			
TG (mg/dL)	**Mean ± SD.**	283.9 ± 80.45		147.4 ± 21.02		U=	<0.001*
	Median	281.0		147.0		19.50*	
	Min. – Max.	169.0 – 470.0		110.0 – 200.0			
HDL (mg/dL)	**Mean ± SD.**	25.69 ± 6.31		29.80 ± 4.24		U=	0.001*
	Median	25.0		30.0		1281.5*	
	Min. – Max.	17.0 – 41.0		21.0 – 40.0			
LDL (mg/dL)	**Mean ± SD.**	156.8 ± 19.35		118.4 ± 16.88		t=	<0.001*
	Median	159.0		120.0		9.706*	
	Min. – Max.	119.0 – 190.0		80.0 – 149.0			

SD.: Standard deviation, Min.: Minimum, Max.: Maximum. X_2: Chi Square, t: Student t test, U: Mann Whitney test, p: Comparing DN and without DN, HbA1c: Glycated hemoglobin, TLC: Total leukocyte count, Hb: hemoglobin, UACR: Urine albumin-creatinine ratio, TG: Triglycerides, HDL: High-density lipoprotein cholesterol, LDL: Low-density lipoprotein cholesterol, * significant as p-value < 0.05.

Table 3: The distribution of SIRT1 rs7069102 gene polymorphism among patients with DN and without DN

SIRT1 rs7069102		DN n = 45		Without DN n = 40		P value	OR (95 % CI)
		No.	**%**	**No.**	**%**		
Genotypes	**CC**	19	42.2	3	7.5		Reference
	CG	21	46.7	21	52.5	0.005*	0.33(0.16–0.71)
	GG	5	11.1	16	40.0	<0.001*	0.16(0.07–0.40)
Dominant model	**CC**	19	42.2	3	7.5		Reference
	CG+GG	26	57.8	37	92.5	<0.001*	0.27(0.13–0.55)
Recessive model	**CC+CG**	40	88.9	24	60.0		Reference
	GG	5	11.1	16	40.0	0.002*	0.36(0.18–0.69)
Alleles	**C**	59	65.6	27	33.75		Reference
	G	31	34.4	53	66.25	<0.001*	0.44(0.30–0.65)

C, Cytosine; G, Guanine; Reference according to NCBI; OR, odds ratio; CI, confidence interval; OR<1 is considered protective; OR>1 is considered risky; *: P value Significant <0.05.

Table 4: Association between SIRT1 rs7069102 gene polymorphism and personal history, HbA1c, CBC, renal function tests, UACR, and lipid profile among patients with DN.

		SIRT1 rs7069102						Test	P
		CC (N = 19)		**GC (N = 21)**		**GG (N = 5)**			
		No.	**%**	**No.**	**%**	**No.**	**%**		
Sex	**Male**	7	36.8	9	42.9	2	40.0	X^2=	MC
	Female	12	63.2	12	57.1	3	60.0	0.286	0.911
Age (years)	**Mean ± SD.**	65.21 ± 6.60		65.57 ± 7.15		64.60 ± 5.41		F=	0.956
	Median	66.0		67.0		65.0		0.045	
	Min. – Max.	53.0 – 77.0		54.0 – 78.0		56.0 – 71.0			
HbA1c %	**Mean ± SD.**	7.82 ± 0.73		7.90 ± 0.67		7.90 ± 0.59		H=	0.949
	Median	7.50		7.90		7.80		0.105	
	Min. – Max.	6.90 – 9.0		6.90 – 8.90		7.20 – 8.50			
TLC ($x10^9$/L)	**Mean ± SD.**	7.23 ± 1.88		7.76 ± 2.44		6.40 ± 2.75		F=	0.449
	Median	7.0		7.80		5.50		0.817	
	Min. – Max.	4.30 – 11.50		4.0 – 13.0		3.50 – 10.0			
Platelet ($x10^9$/L)	**Mean ± SD.**	204.4 ± 47.0		223.6 ± 79.40		164.4 ± 34.34		F=	0.174
	Median	200.0		200.0		175.0		1.826	
	Min. – Max.	90.0 – 300.0		100.0 – 350.0		130.0 – 210.0			
Hb (g/dL)	**Mean ± SD.**	11.87 ± 1.60		12.31 ± 1.58		11.70 ± 1.64		H=	0.498
	Median	11.0		12.0		12.0		1.394	
	Min. – Max.	10.0 – 15.0		10.0 – 15.0		10.0 – 13.50			
Creatinine (mg/dL)	**Mean ± SD.**	4.05 ± 1.32		4.03 ± 0.98		4.88 ± 1.03		F=	0.309
	Median	3.90		4.0		5.0		1.208	
	Min. – Max.	2.0 – 8.0		2.50 – 6.0		3.20 – 6.0			
Urea (mg/dL)	**Mean ± SD.**	121.8 ± 27.31		124.1 ± 25.68		146.4 ± 36.58		F=	0.208
	Median	122.0		124.0		151.0		1.631	
	Min. – Max.	77.0 – 177.0		70.0 – 165.0		100.0 – 185.0			
UACR (mg/g)	**Mean ± SD.**	673.3 ± 162.7		617.6 ± 179.6		729.6 ± 211.9		H=	0.226
	Median	719.0		687.0		835.0		2.972	
	Min. – Max.	370.0 – 898.0		310.0 – 851.0		400.0 – 891.0			
Cholesterol (mg/dL)	**Mean ± SD.**	191.4 ± 41.77		196.7 ± 30.52		182.4 ± 26.70		F=	0.704
	Median	186.0		191.0		195.0		0.354	
	Min. – Max.	119.0 – 290.0		155.0 – 270.0		143.0 – 206.0			
TG (mg/dL)	**Mean ± SD.**	266.2 ± 57.80		285.7 ± 95.0		343.4 ± 71.50		H=	0.234
	Median	279.0		250.0		300.0		2.903	
	Min. – Max.	178.0 – 355.0		169.0 – 470.0		287.0 – 440.0			
HDL (mg/dL)	**Mean ± SD.**	23.42 ± 5.04		27.71 ± 6.26		25.80 ± 9.04		H=	0.132

	Median	21.0	28.0	22.0	4.053	
	Min. – Max.	17.0 – 32.0	17.0 – 41.0	17.0 – 40.0		
LDL (mg/dL)	**Mean ± SD.**	155.7 ± 20.18	159.1 ± 20.78	151.4 ± 7.44	F=	0.695
	Median	157.0	161.0	155.0	0.366	
	Min. – Max.	119.0 – 183.0	129.0 – 190.0	142.0 – 159.0		

SD. Standard deviation, Min.: Minimum, Max.: Maximum, F: One Way ANOVA test. X^2: Chi-Square, MC: Monte Carlo, H: Kruskal Wallis test, P: Comparing the different SIRT1 rs7069102 categories.

Table 5: Logistic regression analysis for prediction of DN among DM patients.

	Univariate			Multivariate		
	P	**OR**	**95% CI**	**P**	**OR**	**95% CI**
Gender	0.813	1.068	0.618–1.847			
Age	0.110	1.027	0.994–1.062			
HbA1c	<0.001*	8.155	3.360–19.792	<0.001*	1.141	1.066-1.222
TLC	0.876	1.009	0.900–1.131			
Platelet	0.269	1.002	0.998–1.006			
Hb	0.398	0.930	0.787–1.100			
Cholesterol	0.736	1.001	0.993–1.009			
TG	<0.001*	1.079	1.035-1.124	<0.001*	1.002	1.001-1.002
HDL	0.001*	0.919	0.873–0.967	0.430	1.003	0.995-1.011
LDL	<0.001*	1.077	1.044–1.112	0.013*	1.003	1.001-1.005
SIRT1 rs7069102	<0.001*	0.268	0.130–0.554	0.001*	0.853	0.775-0.939

OR: Odd Ratio; CI, confidence interval. TLC: Total leukocyte count, Hb: Hemoglobin, TG: Triglycerides, HDL: High-density lipoprotein cholesterol, LDL: Low-density lipoprotein cholesterol, *: Significant when p value <0.05.

Discussion

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by hyperglycaemia and insulin receptor resistance, which burden a risk for the occurrence of micro and macroangiopathic complications, including diabetic nephropathy (DN), which is one of the most severe diabetic complications and the leading cause of end-stage renal disease (ESRD) (10).

Sirtuin 1 (SIRT1), a NAD+-dependent deacetylase involved in various cellular processes including DNA repair, inflammation suppression, and energy metabolism regulation, has been linked to diabetes-related complications. SIRT1 polymorphisms, specifically rs7069102, may influence the development of DN (11).

We conducted cross-sectional study at Benha University Hospital to investigate the relationship between the SIRT1 rs7069102 polymorphism and DN in T2DM patients. The study included 85 subjects, with 45 patients having diabetic nephropathy and 40 patients without nephropathy.

In the current study, demographic comparisons between individuals with DN and those without DN showed no significant differences. The DN group consisted of 18 males (40%) and 27 females (60%), while the non-DN group had 15 males (37.5%) and 25 females (62.5%) (p=0.813). The mean age was 65.31 years in the DN group and 62.43 years in the non-DN group, with no significant difference (p=0.118).

This finding suggests that sex and age may not be significant factors influencing the development of DN in this study population.

However, existing literature on the association between sex and the risk of diabetic kidney disease (DKD) progression presents conflicting findings. Some studies have reported a higher risk of DKD progression in men (12). Conversely, other studies have found a higher risk in women (13-16), while some have reported no significant sexual dimorphism in DKD risk (17). The inconsistencies in these findings underscore the complexity of the relationship between sex and DKD risk. Factors such as genetic predisposition, hormonal influences, and differences in healthcare access and utilization may contribute to these disparities.

In the current study, a comparison of HbA1c levels between individuals with DN and those without DN revealed a highly significant difference. The DN group had a mean HbA1c level of 7.86% (±0.67), while the non-DN group had a mean HbA1c level of 6.94% (±0.35) ($p<0.001$), indicating that individuals with DN had poor glycaemic control compared to those without DN.

Arnold and colleagues. (18) also found a compelling association between baseline HbA1c levels and long-term renal outcomes. Consistent with previous studies (19, 20), subjects with the highest baseline HbA1c levels experienced the most rapid GFR loss and were at the highest risk for new-onset moderate CKD or CKD progression. This observation can be attributed to the transient or constant accumulation of advanced glycation end-products (AGEs), which are stable compounds formed through the nonenzymatic glycation of proteins, amino acids, or lipids. Glycaemic stress increases AGE levels, and the accumulation of specific AGEs has been directly linked to oxidative stress, inflammation, and apoptosis. This process ultimately promotes cellular dysfunction, mediates vascular damage, and contributes to kidney disease (21, 22).

In both the current study and the research conducted by Moosazadeh and co-workers (23), comparisons of CBC parameters between individuals with DN or chronic kidney disease (CKD) and those without these conditions showed no significant differences. In the DN group, the mean TLC was 7.39 (±2.24) × 10^9/L, the mean platelet count was 208.9 (±64.91) × 10^9/L, and the mean haemoglobin level was 12.06 (±1.57) g/dL. In comparison, the non-DN group had a mean TLC of 7.31 (±2.50) × 10^9/L, a mean platelet counts of 191.7 (±79.06) × 10^9/L, and a mean haemoglobin level of 12.35 (±1.65) g/dL. None of these differences were statistically significant, with p-values of 0.878, 0.273, and 0.406, respectively. These findings suggest that CBC parameters may not be indicative of DN or CKD presence, underscoring the need for other diagnostic markers in assessing these conditions.

In the current study, significant differences were observed in renal function tests between individuals with DN and those without DN. The DN group had a mean creatinine level of 4.13 (±1.15) mg/dL, compared to 0.83 (±0.17) mg/dL in the non-DN group, with a highly significant difference ($p<0.001$). Similarly, the DN group had a mean urea level of 125.6 (±27.98) mg/dL, while the non-DN group had a mean of 28.68 (±5.76) mg/dL, also with a highly significant difference ($p<0.001$). These findings indicate a

notable impairment in renal function among individuals with DN.

Anwar et al. (24) supported these findings, emphasizing the prognostic significance of serum urea and creatinine levels in predicting renal impairment in diabetes. Similarly, studies by Bamanikar and others (25) and Ullah and colleagues. (26) confirmed that serum urea and creatinine levels are valuable prognostic markers and predictors of renal damage in diabetic patients.

In the current study, a comparison of the urine albumin-to-creatinine ratio (UACR) between individuals with DN and those without DN revealed significant differences. The DN group had a mean UACR of 653.6 (±176.1) mg/g, while the non-DN group had a mean UACR of 42.38 (±21.77) mg/g. This difference was statistically significant ($p<0.001$), indicating that individuals with DN had considerably higher UACR levels compared to those without DN.

In accordance, Sueud et al. (27) demonstrated that a UACR ratio exceeding 29.8 mg/g predicted the presence of nephropathy with high sensitivity, specificity, and accuracy.

In the comparison of lipid profiles between individuals with DN and those without DN, notable differences were observed. Although there was no significant disparity in cholesterol levels between the two groups ($p=0.740$), individuals with DN exhibited significantly higher levels of triglycerides compared to those without DN (mean of 283.9 ±80.45 mg/dL vs. 147.4 ±21.02 mg/dL, $p<0.001$). Additionally, the DN group had markedly lower levels of HDL cholesterol compared to the non-DN group (mean of 25.69 ±6.31 mg/dL vs. 29.80 ±4.24 mg/dL, $p=0.001$). Moreover, individuals with DN demonstrated significantly higher levels of LDL cholesterol compared to those without DN (mean of 156.8 ±19.35 mg/dL vs. 118.4 ±16.88 mg/dL, $p<0.001$). These findings indicate that DN is associated with unfavourable changes in lipid profiles, characterized by elevated triglyceride and LDL cholesterol levels, and reduced HDL cholesterol levels.

Palazhy and Viswanathan (28) noted a significantly higher prevalence of dyslipidaemia among individuals with nephropathy in their study. They also observed significantly higher levels of total cholesterol, triglycerides, and LDL-C among nephropathy patients. Suchitra et al. (29) also reported significant differences in total cholesterol, triglycerides, HDL-C, and LDL-C between diabetic and diabetic nephropathy patients. These findings align with studies conducted in different ethnic populations, reinforcing the observed associations (30).

In our study, differences distinctions emerged in the distribution of the SIRT1 rs7069102 gene polymorphism among patients with and without DN. Specifically, the CC genotype was notably more prevalent in patients with DN compared to those without DN (42.2% vs. 7.5%). Conversely, the GC genotype was significantly higher in patients without DN (52.5% vs. 46.7%, $p=0.005$), as was the GG genotype (40.0% vs. 11.1%, $p<0.001$). Further analysis using dominant and recessive models bolstered these findings, with combined GC+GG genotypes significantly associated with being without DN (92.5% vs. 57.8%, $p<0.001$), and the GG genotype significantly associated with not having DN (40% vs. 11.1%, $p=0.002$). Moreover, the G allele frequency was significantly higher in patients without DN (66.25% vs. 34.4%, $p<0.001$). Odds ratios

for CG, GG genotypes, dominant and recessive models, and the G allele were less than 1, indicating a protective effect against DN occurrence. These results imply an association between SIRT1 rs7069102 gene polymorphism and the risk of developing DN, with certain genotypes and alleles potentially conferring protection against the disease.

Letonja et al. (11) similarly found an association between the C allele of SIRT1 rs7069102 polymorphism and DN in patients with T2DM ($p = 0.01$). They employed linear regression to statistically determine the relationship between rs7069102 genotypes and DN, revealing that patients with the CC genotype are more likely to develop DN than patients with other genotypes. Utilizing a codominant model of inheritance, they found that patients with the CC genotype were 1.94 times more likely to develop DN ($p = 0.02$) than those with other genotypes. Additionally, the application of a recessive model of inheritance indicated that T2DM patients with the CC genotype are 2.39 times more likely to develop DN than those with the CG or GG genotype ($p = 0.02$). However, the dominant model of inheritance did not show a significant correlation of the CC and CG genotype with DN when compared to the GG genotype. SIRT1's involvement in various cellular mechanisms implicated in DN pathogenesis suggests that the rs7069102 polymorphism could affect these mechanisms.

In a study by Khalil et al. (10), significant differences were noted in the genotype frequency and allelic distribution of SIRT1 rs7069102, with the CC genotype and C allele being more prevalent in the DN group, indicating an increased risk for developing DN.

Priya et al. (31) explored various polymorphisms of the SIRT1 gene and their association with DN. They found that the GA genotype of the rs10823108 polymorphism in the SIRT1 gene is associated with a 35% increased risk of DN in women compared to those with T2DM. Additionally, serum Sirtuin 1 levels were elevated in both DN and T2DM patients compared to control individuals. Additionally, studies in the Chinese population by Zhao et al. (32) and Yue et al. (33) investigated the correlation between SIRT1 polymorphisms and DN, with both reinforcing our findings and Yue et al. (33) describing the protective role of another SIRT1 polymorphism rs3818292 against DN.

In our current study, no significant associations were observed between the SIRT1 rs7069102 gene polymorphism and demographic factors, HbA1c levels, CBC parameters, renal function tests, UACR, or lipid profile parameters in either the DN group or the non-DN group.

However, logistic regression analysis aimed at predicting DN in patients with DM highlighted several key factors. In univariable analysis, higher HbA1c levels, TG, LDL, lower HDL, and the absence of the CC or GG genotypes of SIRT1 rs7069102 were associated with DN. Nevertheless, upon conducting multivariable analysis, only higher HbA1c, TG, LDL, and the absence of the CC or GG genotypes of SIRT1 rs7069102 remained significant independent predictors of DN. This suggests a stronger association of these factors with the development of DN, while other factors did not maintain independent predictive value when considered collectively.

Supporting this, Lian and colleagues (34) and Lind and colleagues (35) confirmed

the role of HbA1c in predicting DN. Yang and Jiang (36) also identified glycosylated haemoglobin A1c (HbA1c) and triglycerides (TG) as predictors for DN. Additionally, Letonja and colleagues (11) found that the CC genotype of the rs7069102 polymorphism in SIRT1 was a predictor of diabetic kidney disease (DKD).

Conclusions:

The study highlighted the significant association between the SIRT1 rs7069102 polymorphism and the risk of diabetic nephropathy in patients with T2DM. SIRT1 rs7069102 was found to be an independent predictor of DN occurrence in those patients. Further research is needed to validate these associations in diverse populations and investigate whether this variant is associated with other complications related to diabetes.

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