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Association of Silent Information Regulator 1 (Sirt1) Gene Polymorphism with the Pathogenesis of Diabetic Nephropathy.

Ola Abdelmoneim ElKholy [1], Gihan Farouk Attia [2], Wesam Salah Ibrahim [2], Amal Selim [3]

[1] Clinical Pathologist in General authority for health, Cairo, Egypt

[2] Clinical Pathology Department, Faculty of Medicine, Tanta University, Tanta, Egypt

[3] Internal Medicine Department, Faculty of Medicine, Tanta University, Tanta, Egypt

Abstract

Introduction: Diabetic nephropathy (DN) is one of the major microvascular complications of diabetes mellitus. Patients with diabetic nephropathy are at a higher risk of morbidity and mortality than those without nephropathy. In this case, early diagnosis and prevention of DM are crucial. SIRT1, which is among the seven members of sirtuins, is recognized as an important element in the pathogenesis of type 2 diabetes, therefore it has several actions in the diabetic nephropathy that will be further discussed in this study. This study will analyse the relationship between SIRT1 gene polymorphism and serum sirt1 in the pathogenesis of diabetic nephropathy.

Aim of the study: evaluation of the association of Silent Information Regulator1 (SIRT1) gene polymorphism and serum SIRT1 protein with type 2 diabetic patients and their role in the pathogenesis of diabetic nephropathy.

Subjects and Methods: This study was carried out on 120 subjects with matched age and sex. They were divided into 3 groups: (Group 1: 40 diabetic nephropathy patients, Group 2: 40 diabetic patients without diabetic nephropathy, Group 3: 40 healthy control subjects) to discuss the association of sirt1 gene polymorphism and the pathogenesis of diabetic nephropathy.

Results: The mean levels of the serum SIRT1 protein was significantly increasing in the diabetic nephropathy group than the diabetics without diabetic nephropathy and the controls, while it was not significant between the diabetics without DN and the control group.

Conclusion: Based on this study, SIRT1 gene polymorphism is a significant factor in the development and progression of DN.

Keywords: Diabetic Nephropathy – SIRT1 gene – Pathogenesis – Kidney

Introduction

Diabetic nephropathy (DN) is one of the major microvascular complications of diabetes mellitus and occurs in approximately 30% of type 2 D.M. patients **(1).** DN is the leading cause of end-stage renal disease and a major risk factor for cardiovascular disease **(2).**

Patients with diabetic nephropathy are at a higher risk of morbidity and mortality than those without nephropathy. In this case, early diagnosis and prevention of DM are crucial **(3).**

Mounting evidence has showed that the pathogenesis of DM was associated partially with a prolonged duration or inadequate metabolic and / or blood pressure control in some cases. However, a clinically significant phenomenon can be observed that even diabetic individuals with excellent blood glucose control may still develop renal complications **(4).**

SIRT1, which is among the seven members of sirtuins, is recognized as an important element in the pathogenesis of type 2 diabetes **(5).**

Recent studies of SIRT1 in relation to kidney disorders have demonstrated its efficacy for nephroprotection **(6).**

It was reported that 4 independent nucleotide polymorphisms (SNPs) present in SIRT1 have been linked to diabetic kidney disease in Japanese type2 diabetics. Utilizing a bioinformatics method revealed that only one SNP (rs 4746720) is present in the 3 UTR area, while the other 3 are located in the intron area. This examination implies that SIRT1 (rs 4746720) could be a candidate gene for vulnerability to diabetic kidney disease **(7).**
This study will examine the relationship between SIRT1 gene polymorphism and serum sirt1 in the pathogenesis of diabetic nephropathy.

Diabetic Nephropathy

DN is a chronic, progressive disease of the kidney that develops over time, with a peak incidence after 10– 20 years of diabetes. **(8).**

Pathogenesis:

Uncontrolled D.M. for long periods leads to damage and disruption of the renal cellular architecture and microvasculature of diabetic patients. The pathways that mediate these effects are grouped into four main categories: metabolic, hemodynamic, intracellular, and growth factors/cytokines. **(9, 10)**.

The pathogenesis of DN is multifactorial, involving a complex series of molecular processes. The pathological changes of DN are composed of histopathological and functional changes which interact with each other **(11).**

- **Hemodynamic Alternations**

DN is characterized at its onset by glomerular hyperfiltration. Potential mechanisms leading to glomerular hyperfiltration include a combination of hemodynamic, vasoactive, tubular, growth-promoting, and metabolic factors. **(12, 13)**.

- **Metabolic Pathways:**

Glucose can metabolize to sorbitol and accumulate in mesangial cells, which leads to nicotinamide adenine dinucleotide phosphate depletion, decreased nitric oxide (NO), increased oxidative stress, and activation of protein kinase C (PKC). Phosphorylation of PKC then activates pathways of transforming growth factor-β (TGF-β) and vascular endothelial growth factor (VEGF), as well as reactive oxygen species (ROS) and angiotensin II. This causes mesangial expansion and induce cell injury. **(14).**

- **Histopathological Changes**:

DN is characterized by a constellation of histopathological changes including glomerular basement membrane (GBM) thickening, mesangial expansion (in early DN), Kimmelstiel–Wilson nodules (an aggregation of mesangial cells and mesangial matrix), arterial hyalinosis, and tubulointerstitial changes (e.g., fibrosis and tubular atrophy in advanced DN). **(15, 16)**

Podocyte dropout is also a critical factor for DN development. Since, podocytes are not readily replaced, the remaining podocytes change their size and shape to cover the portion of the GBM left ‗naked' by lost podocytes. **(15, 17).**

- **Inflammatory Pathways:**

Macrophage accumulation in kidneys of patients with diabetes predicts the decline of renal function, suggesting a pathogenic role for these cells in DN. **(18, 19, 20)**.

Hyperglycemia can induce macrophage production of (IL)-12, which can stimulate (IFN-γ) production by CD4 cells. Free fatty acids, hyperglycemia, and obesity may activate (NF-kB) and allow NF-kB translocation to the nucleus, which subsequently stimulates transcription of genes such as those related to endothelin-1, vascular cell adhesion molecule-1, ICAM-1, IL-6, and tumor necrosis factor-α (TNF-α) that promote the development of DN. **(21, 22)**.

- **Role of the Renin–Angiotensin Aldosterone System:**

Angiotensin II can activate and upregulate NF-kB, causing production of chemokines and further renal damage. **(23)**.

Angiotensin II is also localized in tubular-, interstitial-, and fibroblast-like cells, which work together with high glucose and inflammatory mediators to target tubular cells and cause impaired kidney function in diabetes, by hypertrophy of mesangial cells and tubular epithelial cells and promote transforming growth factor-B (TGF-β) production, which can cause glomerular sclerosis. **(11)**

- **Endothelial Dysfunction as a Potential Contributor:**

Advanced diabetic glomerulopathy often exhibits thrombotic microangiopathy, including glomerular capillary microaneurysms and mesangiolysis, which are typical manifestations of endothelial dysfunction in the glomerulus. **(24)**.

Hyperglycemia cause reduction in the NO levels in the endothelium and contribute to the development of endothelial dysfunction. **(24)**.

Clinical Staging of Diabetic Nephropathy(25, 26)

Stage	GFR (mL/ min/1.73 m^2)	AER (mg/g creatinine)	Duration of diabetes (years)
Hyperfiltration	>120	<30	0–5
Microalbuminuria	Normal	30–300	5–15
Macroalbuminuria/ proteinuria	Normal or <90	>300	10–20
Progressive kidney disease	Normal or <90	>3000	15–25
End-stage renal disease	<15	>3000	20–10

Clinical Assessment of Diabetic Nephropathy

Clinically, DN is classically characterized by progressive increases in urinary albumin excretion (UAE). This is accompanied by a gradual decline in glomerular filtration rate (GFR) and eventual progression to ESRD. Defects at the level of the GFB leads toward increased urinary protein. **(27)**.

The degree of albuminuria and proteinuria correlates with and is also an important clinical predictor of the rate of kidney disease progression. **(28)**.

Estimation of GFR is an important clinical investigation in monitoring renal function decline. Routine annual surveillance is recommended for all diabetic patients to monitor the progression and rate of decline of renal function. **(29).**

Silent Information Regulator 1 (SIRT1)

Sirtuins are known as the NAD+ - dependent protein deacetylases, which have effects against diseases that are related to age for instance diabetes, cancer, neurodegenerative, cardiovascular & renal diseases. **(30)**

Structure and Distribution of SIRT1:

In mammals, the sirtuin family is the homolog of the Sir2 gene, consisting of 7 isoforms. **(31).**
All of them possessing a highly conserved central NAD+ - binding site and common catalytic domain. **(32)**

The 7 isoforms have the same 275-amino-acid-sized catalytic core region and a diverse subcellular localization SIRT1 is the most extensively studied family member. **(31)**

SIRT1, SIRT6 and SIRT7 are mainly found in the nucleus, and SIRT2 is in the cytoplasm, while SIRT3, SIRT4 and SIRT5 are located in the mitochondria. **(33)**.

The human SIRT1 gene is located on chromosome 10q22.1. This gene includes nine exons and eight introns and is approximately 33kb long. This gene is widely expressed in foetal and adult tissues, including fat and muscle tissues of the liver, kidneys, and brain. It is also uniformly expressed in islet cells but is rarely expressed in islet exocrine gland cells. **(34).**

Biological Effects of SIRT1:

SIRT1 mainly utilizes deacetylase activity to exert its regulatory effects on various physiological processes, including gene transcription, energy metabolism, cell senescence, glucose metabolism, lipid metabolism, and insulin secretion **(35)**

In the kidney, SIRT1 promotes podocyte function and mitochondrial biogenesis, and inhibits fibrosis, inflammation, and apoptosis. **(36, 34, 37)**

SIRT1 in Health and Diseases:- ✓

SIRT1 and glucose metabolism:

The Sirtuins generaly and SIRT1 particulary influence glucose metabolism in liver, muscle, adipose tissue, and pancreas. **(38).**

The SIRT1 overexpression is associated with decrease in serum insulin and cholesterol along with a decrease in adipose tissue and reduction of obesity-induced insulin resistance. **(39).**

✓ **SIRT1 and development:**

High levels of SIRT1 mRNA are detectable in brain, heart, spinal cord & dorsal root ganglia of embryos, indicating a vital role in cell development **(40).**

The Association of SIRT1 gene in the Pathogenesis of Diabetic Nephropathy in type 2 diabetics **SIRT1 actions in diabetic nephropathy(41, 42).**

I. SIRT1 Preserves Podocyte Function.
II. SIRT1 Reduces Fibrosis by Smad3 and Smad4.
III. SIRT1 Inhibits Apoptosis by Targeting p53, Smad7, FOXO3 and FOXO4.
IV. SIRT1 Suppresses Inflammation by Targeting NF-κB and High Mobility Group Box 1(HMGB1). **V.** SIRT1 Induces Autophagy by Targeting Autophagy-Related Genes and FOXO3.
VI. SIRT1 Regulates Blood Pressure by Targeting Endothelial Nitric Oxide Synthase and Angiotensin II Type 1 Receptors.
VII. SIRT1 Enhances Mitochondrial Biogenesis by Targeting Peroxisome Proliferator–Activated Receptorγ Coactivator 1α.
VIII. SIRT1 Modulates Hypoxic Responses by Targeting Hypoxia-Inducible Factor-1a (HIF-1a) and (HIF-2a).
IX. SIRT1 Regulates Metabolism by Targeting Sterol Regulatory Element Binding Protein, Liver X Receptor, Nuclear Bile Acid Receptor and Insulin Receptor Substrate-2.

Subjects and Methods

Subjects:

This study was carried out on type 2 diabetic patients with and without diabetic nephropathy and healthy control individuals with matched age and sex.

The subjects were divided into three groups:

***Group I:** it consisted of 40 type 2 diabetic patients suffering from diabetic nephropathy. They were 16 males and 24 females. Their ages ranged from 53 to 65 years.

***Group II:** it consisted of 40 type 2 diabetic patients without diabetic nephropathy. They were 24 males and 16 females. Their ages ranged from 52 to 68 years.

***Group III:** it consisted of 40 normal healthy individuals as a control group. They were 16 males and 24 females. Their ages ranged from 50 to 64 years.

All studied cases will be subjected to:

- **Full history taking:**

(age, sex, diabetes, hypertension, duration of diabetes, duration of hypertension).

- **A. Routine laboratory investigation:**

− Fasting and postprandial blood glucose level.
− Glycosylated haemoglobin (HbA1c).
− Serum urea and creatinine level.
− Blood Urea Nitrogen (BUN).
− Microalbuminuria.
− Urinary Albumin / Creatinine ratio**.**

- **B. Specific laboratory investigation:**

− Screening of Single Nucleotide Polymorphism (SNP) genotyping SIRT1 polymorphism (rs4746720) using real time PCR in the studied groups.
− Serum Sirt1 Protein was measured by commercial sandwich ELISA Kits.

Methods:

I. Measurement of Serum Level of SIRT1:

Enzyme-linked immunosorbent assay was used to measure the SIRT1 protein levels in the three studied groups. ***(43)***

Using commercial Enzyme-linked Immunosorbent Assay Kit For the quantitative detection of SIRT1, supplied by Clini-lab catalog no.: MBS2503120. ***(43)***

II. *Detection of SIRT1 gene polymorphism (rs4746720):*

- **DNA Analysis: was carried out in the three groups of the study.**
- **DNA extraction protocol:**

DNA was isolated from the peripheral frozen whole blood. **(44)** Using DNA extraction kits (GeneJET Genomic DNA Purification Mini Kits) supplied by Clini-lab.

Procedure:

1. 400 µL of Lysis Solution and 20 µL of Proteinase K Solution were added to 200 µL of whole blood, and mixed thoroughly by vortexing.
2. The sample was incubated at 56°C and mixed by vortexing occasionally.
3. 200 µL of ethanol (96%-100%) was added and mixed by pipetting and vortexing.
4. The prepared lysate to a GeneJET Genomic DNA Purification Column was transferred and inserted in a collection tube. The column was centrifuged for 1 min at 6000 x g, and the collection tube containing the flow-through solution was discarded. The GeneJET Genomic DNA Purification Column was placed into a new 2 mL collection tube (included).
5. 500 µL of Wash Buffer I was added (with ethanol added). Centrifuged for 1 min at 8000 x g. The flowthrough was discarded and the purification column back placed into the collection tube.

6. 500 µL of Wash Buffer II was added (with ethanol added) to the GeneJET Genomic DNA Purification Column. Centrifuged for 3 min at maximum speed (≥12000 x g).
7. 200 µL of Elution Buffer was added to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA. Incubated for 2 min at room temperature and centrifuged for 1 min at 8000 x g.
8. The purification column was discarding. The purified DNA was used immediately in downstream applications or stored at -20 °C.

DNA concentration and purity:

The concentration of DNA was determined from the elute by diluting with nuclease free water 1:50 then measured absorbance (A) in spectrophotometer.

Calculation: DNA solution at a concentration of 50 µg/mL provides an absorbance of 1.0 at 260 nm Therefore the absorbance multiplied by 50 provides the concentration of the diluted DNA solution and multiplied by factor dilution (50) provides the concentration of the original DNA in µg/mL= Absorbance reading at 260 nm /2500.

The DNA pureness was determined by calculating the ratio of the absorbance at 260 nm to absorbance at 280 nm. Pure DNA had an A 260/A 280 ratio of 1.7-2.0.

The DNA extracted was stored at -20 °C until further processing.

III. <u>Real Time PCR for SIRT1 polymorphism</u>:

SNP **rs4746720** of mammalian Silent Information Regulator 1 (SIRT1) gene determined by using real time PCR. **(44)** By using real time PCR kit (Applied bio system, step 1 version).

Principle:

Allelic discrimination assays, the PCR assay includes a specific fluorescent dye labeled 2 probes for each allele. The probes have different fluorescent reporter dyes (FAM and VIC) to discriminate the amplification of each allele.

During PCR, each probe combined specifically to complemental sequences between the forward and reverse primer sites. AmpliTaq Gold DNA polymerase can slice only probes that hybridize to the allele. Cleavage separates the reporter dye from the quencher dye. This results in increased fluorescence by the reporter dye. Thus, the fluorescence signal(s) made by PCR amplification show(s) the alleles that are present in the sample.

Additionally, AmpliTaq Gold DNA polymerase is more likely to displace the mismatched probe rather than cleave it to show reporter dye.

Two probes contain a non-fluorescent quencher at the 3‘ end. Because this quencher does not fluoresce, the Sequence Detection Systems instruments measure the reporter dye contributions more perfect.

Procedure:

Reagents:

1) 40µ SNP genotyping assay which have:

• Specific-sequence to amplify the sequence of interest **rs4746720**

- ✓ Forward primer **rs4746720**F: TGCTGGCCTAATAGAGTGGCA
- ✓ Reverse primer **rs4746720**R: CTCAGCGCCATGGAAAATGT

• Two TaqMan® MGB probes: One probe labeled with VIC® dye detects the Allele C Sequence and one probe labeled with FAM™ dye detects the Allele T Sequence.

2) TaqMan® Universal PCR Master Mix:

Negative Control: was run with the samples. The DNA template was replaced with H_2O PCR grade.

Statistical analysis:

Collected data was organized, tabulated and statistically analyzed using SPSS software statistical computer package version 12. For quantitative data, the range, mean and standard deviation were calculated. Chisquare was used as a test of significance. Significance was adopted at $p<0.05$ for interpretation of results of tests of significance.

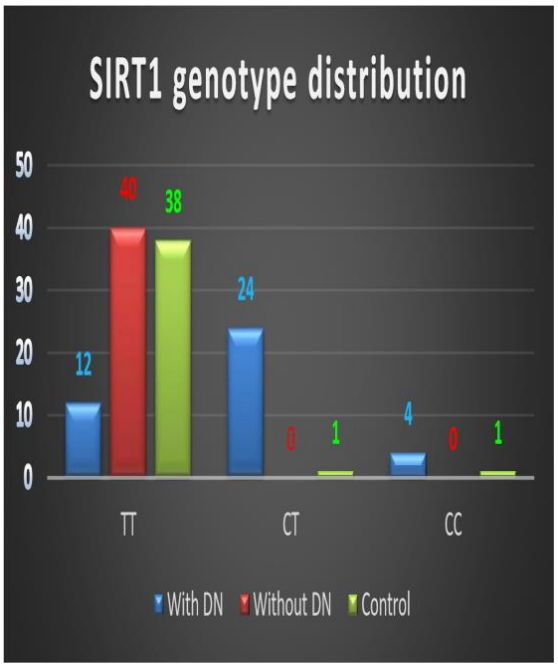
Results

Comparison between the studied groups regarding the following parameters:

		Range (mmHg)	Mean± S. D	F. test	p. value		
Systolic Blood Pressure	**Gp I**	105 – 150	126.50± 15.20	12.895	0.001*	P1	0.001*
	Gp II	110 – 120	117.00 ± 4.05			P2	0.001*
	Gp III	115 - 120	118.00 ± 2.45			P3	0.186
		Range (mmHg)	**Mean ± S.D**	**F. test**	**p. value**		
Diastolic Blood Pressure	**Gp I**	90 - 110	98.00±9.92	138.91	0.001*	P1	0.001*
	Gp II	75 - 80	77.90±2.28			P2	0.001*
	Gp III	70 - 80	78.50± 3.05			P3	0.322
		Range (mg/dL)	**Mean ± S. D**	**F. test**	**p. value**		
Fasting Blood Glucose Level	**Gp I**	160 – 320	217.50 ± 50.40	163.542	0.001*	P1	0.001*
	GpII	150 – 220	186.40 ± 24.78			P2	0.001*
	Gp III	80 - 105	90.00 ± 8.46			P3	0.001*
		Range (mg/dL)	**Mean ± S. D**	**F. test**	**p. value**		
Post Prandial Blood Glucose Level	**Gp I**	210 – 405	301.50 ± 67.41	247.084	0.001*	P1	0.002*
	Gp II	230 – 290	266.00 ± 20.85			P2	0.001*
	Gp III	105 – 125	110.00 ± 7.75			P3	0.001*
		Range (%)	**Mean± S. D**	**F. test**	**p. value**		
HbA1c	**Gp I**	7.5 - 10.5	8.82 ± 0.81	466.596	0.001*	P1	0.001*
	Gp II	6.9 - 9	7.86 ± 0.85			P2	0.001*
	Gp III	3.9 – 4.7	4.30 ± 0.29			P3	0.001*
		Range (mg/dL)	**Mean± S. D**	**F. test**	**p. value**		
Serum Urea Level	**Gp I**	47 - 75	56.75 ± 8.92	517.885	0.001*	P1	0.001*
	Gp II	15 - 27	21.60 ± 4.33			P2	0.001*
	Gp III	12 - 20	17.90 ± 2.47			P3	0.001*
		Range (mg/dL)	**Mean ± S. D**	**F. test**	**p. value**		
Blood Urea Nitrogen (BUN)	**Gp I**	21.96 - 35.05	26.52 ± 4.17	517.885	0.001*	P1	0.001*
	Gp II	7.01 - 12.62	10.09 ± 2.02			P2	0.001*
	Gp III	5.61 - 9.35	8.36 ± 1.15			P3	0.001*
		Range (mg/dL)	Mean ± S. D	F. test	p. value		
Serum creatinine	**Gp I**	1.5 – 2.4	1.75 ± 0.24	786.068	0.001*	P1	0.001*
	Gp II	0.4 – 0.7	0.56 ± 0.10			P2	0.001*
	Gp III	0.3 – 0.6	0.46 ± 0.10			P3	0.004*
		Range (mg albumin / gm creatinine)	**Mean± S. D**	**F. test**	**p. value**		
ACR	**Gp I**	35 – 175	111.85 ± 52.72	156.213	0.001*	P1	0.001*
	Gp II	8 – 20	14.20 ± 4.64			P2	0.001*
	Gp III	1 – 2	1.50 ± 0.35			P3	0.001*
		Range (mg/L)	**Mean± S. D**	**F. test**	**p. value**		
Microalbumin urea	**Gp I**	35 – 185	113.10 ± 54.30	121.371	0.001*	P1	0.001*
	Gp II	20 – 25	22.60 ± 1.8			P2	0.001*
	Gp III	8 – 20	14.20 ± 4.64			P3	0.001*
		Range (ng/µl)	**Mean± S. D**	**F. test**	**p.value**		
Serum SIRT1 protein	**Gp I**	6.3 – 25	15.18 ± 6.46	135.601	0.001*	P1	0.001*
	Gp II	1 – 5	2.76 ± 0.82			P2	0.001*
	Gp III	1 – 5	2.99 ± 1.088			P3	0.285

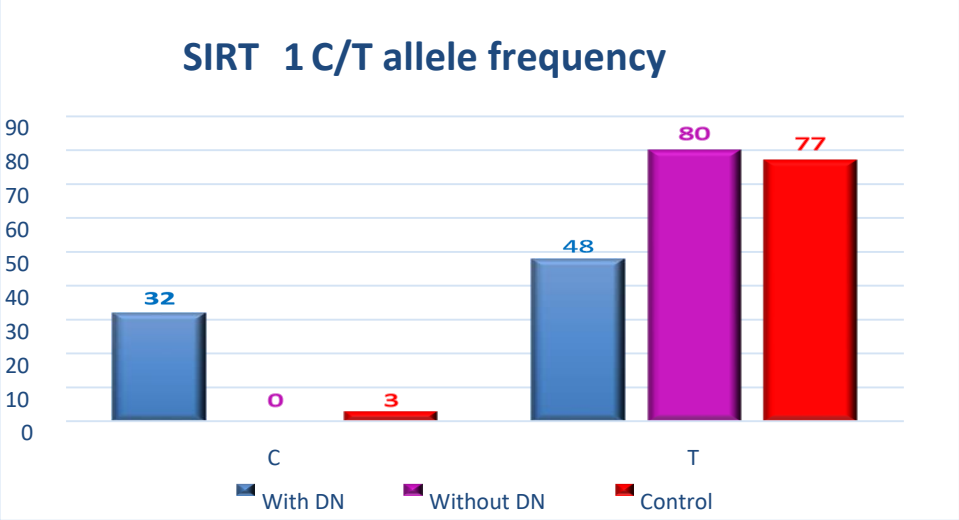
Statistical comparison between the studied groups regarding the SIRT1 genotype distribution:

		Gp I	Gp II	Gp III	X2		
TT	**N**	12	40	38	43.08	**P1**	0.001*
	%	30%	100 %	95%			
CT	**N**	24	0	1	36.48	**P2**	0.001*
	%	60%	0%	2.5%			
CC	**N**	4	0	1	2.05	**P3**	0.359
	%	10%	0%	2.5%			
Total	**N**	40	40	40	–		–
	%	100 %	100 %	100%			
Chi-Square	**X2**	65.72					
	P-value	0.001*					



Comparison between the three studied groups regarding SIRT1C/T allele frequency:

	Gp I (n=40)		Gp II (n=40)		Gp III (n=40)				
	N	**%**	**N**	**%**	**N**	**%**	**X^2**		
C	32	40	0	0	3	3.75	40	**P1**	0.001*
T	48	60	80	100	77	96.25	30.76	**P2**	0.001*
Total	80	100	80	100	80	100	3.06	**P3**	0.0804
Chi-Square	**X2**	62.684							
	P-value	0.001*							



Comparison between the studied parameters in different SIRT1 genotypes in DN group:

HbA1c	Genotypes		
	TT	CT	CC
Range	8.70-9.30%	7.50-8.10%	10.50%
Mean ± SD	7.83% ± 0.002	9.03% ± 0.002	10.50% ± 0
f-test	264.990		
P-value	0.001*		
	TT&CT	TT&CC	CT&CC
t-test	13.9996	36.456	35.668
P-value	0.001*	0.001*	0.001*

Serum Urea	Genotypes		
	TT	CT	CC
Range	47-50	47-75	70
Mean ± SD	48 ± 1.414	58.92 ± 7.702	70 ± 0
f-test	22.152		
P-value	0.001*		
	TT&CT	TT&CC	CT&CC
t-test	6.570	51.595	6.901
P-value	0.001*	0.001*	0.001*

Blood Urea Nitrogen (BUN)	Genotypes		
	TT	CT	CC
Range	21.96-23.36	21.96-35.05	32.71
Mean ± SD	22.43 ± 0.661	27.53 ± 3.599	32.71 ± 0
f-test	22.152		
P-value	0.001*		
	TT&CT	TT&CC	CT&CC
t-test	6.570	51.595	6.901
P-value	0.001*	0.001*	0.001*

Serum Creatinine	Genotypes		
	TT	CT	CC
Range	1.5-2.2	1.5-2.2	1.8
Mean ± SD	1.76 ± 0.312	1.75 ± 0.216	1.8 ± 0
f-test	0.084		
P-value	0.919		
	TT&CT	TT&CC	CT&CC
t-test	0.120	0.443	1.225
P-value	0.453	0.333	0.117

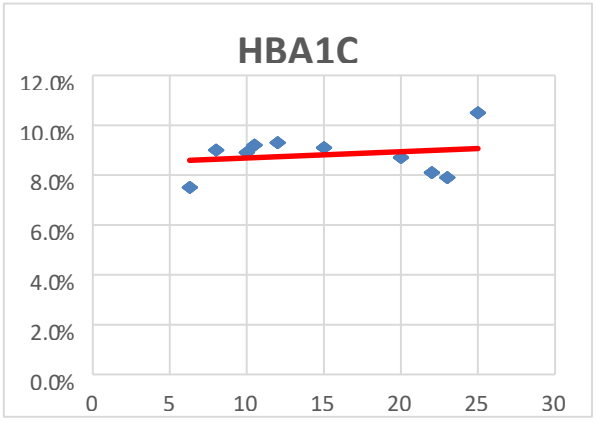
Albumin / Creatinine ratio	Genotypes		
	TT	CT	CC
Range	35-95	56-175	170
Mean ± SD	56.67 ± 27.18	129.75± 42.60	170 ± 0
f-test	21.109		
P-value	0.001*		
	TT&CT	TT&CC	CT&CC
t-test	6.115	13.828	4.628
P-value	0.001*	0.011*	0.0002*

Microalbuminurea	Genotypes		
	TT	CT	CC
Range	35-95	56-185	170
Mean ± SD	56.67 ± 27.18	131.83± 44.03	170 ± 0
f-test	19.903		
P-value	0.001*		
	TT&CT	TT&CC	CT&CC
t-test	6.108	13.828	4.157
P-value	0.001*	0.011*	0.0002*

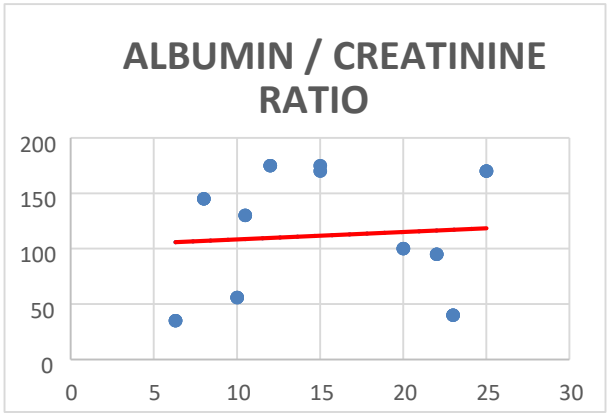
SIRT1	Genotype		
	TT	CT	CC
Range	6.3 - 23	8 - 20	25
Mean ± SD	17.1 ± 7.65	12.6 ± 3.94	25 ± 0
f-test	10.187		
P-value	0.001*		
	TT & CT	TT & CC	CT & CC
t-test	1.845	3.426	15.108
P-value	0.043*	0.002*	0.001*

Correlation between SIRT1 level and the following studied parameters:

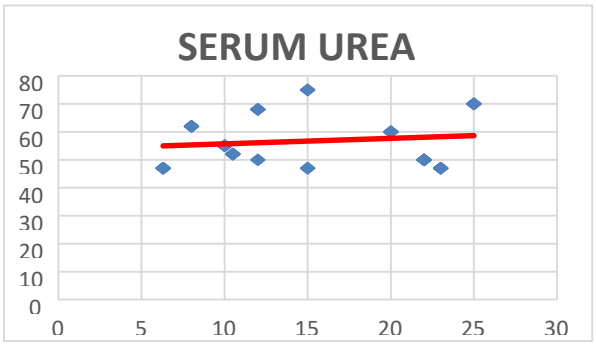
	SIRT1	
	r	p
HbA1c	0.153	0.345
Serum Urea	0.107	0.510
Blood Urea Nitrogen (BUN)	0.107	0.510
Serum Creatinine	0.056	0.728
Albumin/Creatinine ratio	0.081	0.616
Microalbuminurea	0.029	0.861



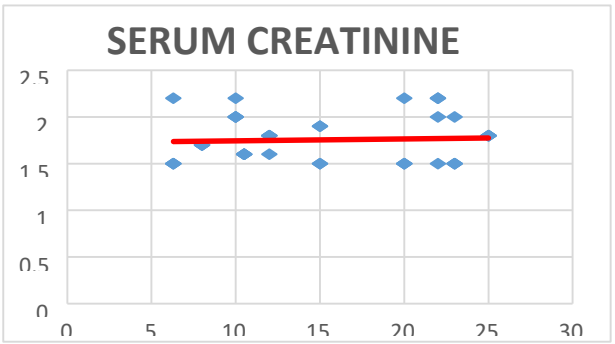
Correlation between SIRT1 level and Glycated Hemoglobin



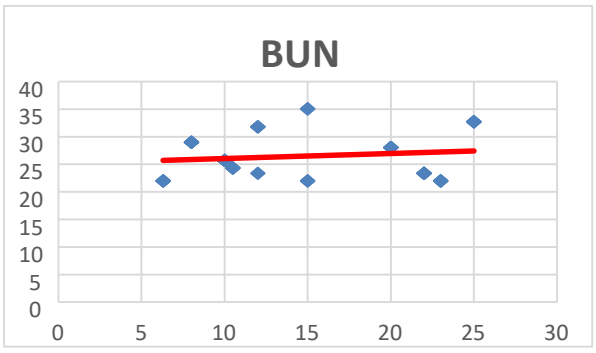
Correlation between SIRT1 level and Albumin/Creatinine ratio



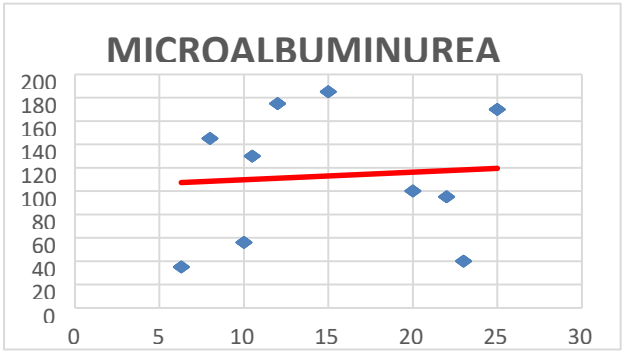
Correlation between SIRT1 level and Serum Urea



Correlation between SIRT1 level and Serum Creatinine



Correlation between SIRT1 level and Blood Urea Nitrogen



Correlation between SIRT1 level and Microalbuminurea

Discussion

There was significant increase difference between the DN group and the diabetics without DN and the control groups, as well as there was a significant increase difference between the diabetics without DN and the controls, regarding the glycated hemoglobin. This is in agreement with a study done by **(45)** between T2DN group and the control group (T2DM without DN) and with the Chinese study done by **(44)** between the DKD group and the control group (DM without DKD) regarding this parameter.

In the present study, there was significant increase difference between the DN group and the diabetics without DN and the control groups, as well as there was a significant increase difference between the diabetics without DN and the controls, regarding the albumin/creatinine ratio, and the microalbuminurea. This is in agreement with the results revealed by **(44)**, in which there was a significant difference between DKD group and the control group (DM without DKD) regarding these parameters

This study stated that there was a significant increase difference in the serum SIRT1 level in the diabetic nephropathy group than the diabetics without DN and the control groups, while there was no significant difference between the diabetics without DN and the control group regarding this parameter.

Regarding the genotype variants of SIRT-1 (rs4746720) distribution. This current study showed that the percentage of TT genotype were significantly decreased in the diabetic nephropathy patients than the diabetic patients without diabetic nephropathy & the control ones. While, the percentage of CT & CC genotypes was significantly increased in the DN patients than the diabetic patients without DN & the control group. In addition that there was no significant difference between the diabetics without DN and the control group regarding the percentage of TT, CT, & CC genotypes. Moreover comparing allele distributions between DN patients, diabetics without DN and normal subjects, this work revealed significantly decreased frequencies of the T allele in DN patients than in diabetics without DN and controls.

Regarding the allele frequency of SIRT-1 (rs4746720). This current study showed that the percentages of T allele were significantly decreased in the diabetic nephropathy patients than the diabetic patients without diabetic nephropathy & the control ones. While, the percentages of C allele were significantly increased in the DN patients than the diabetic patients without DN & the control group. In addition that there was no significant difference between the diabetics without DN and the control group regarding the percentages C and T alleles.

This study showed that the HbA1c, Serum Urea, Blood Urea Nitrogen, levels were statistically significantly increase in DN patients with SIRT-1 CT and CC genotype than those with SIRT-1 T/T genotype.

Adding to this, the Albumin/creatinine ratio and Microalbuminurea levels were statistically significantly increase in DN patients with SIRT-1 CT and CC genotype than those with SIRT-1 T/T genotype.

In this study, the Serum Creatinine level was statistically insignificantly increase in DN patients with SIRT-1 CT and CC genotype than those with SIRT-1 T/T genotype.

So from the previous results, SIRT1 rs4746720 was associated with poor/worse diabetic nephropathy, as manifested by significantly increase HbA1c, Serum Urea, Blood Urea Nitrogen, Albumin/creatinine ratio and Microalbuminurea levels and insignificantly increase level of serum creatinine in CT and CC genotypes. Such findings highlight the usefulness of SIRT1 gene polymorphisms as potential prognostic marker in patients with diabetic nephropathy.

This study showed that the Serum SIRT-1 levels were statistically significantly increased in DN patients with SIRT1 CC genotype than those with SIRT-1 CT and TT genotypes.

The results obtained from this study showed significantly increased serum SIRT-1 levels in diabetic nephropathy group compared to the diabetics without DN and control groups. Which were positively correlated with HbA1c, Serum Urea, Blood Urea Nitrogen, Serum creatinine, Microalbuminurea levels, and Albumin/ creatinine ratios in DN patients.

Conclusion & Recommendations

After studying the association of SIRT1 gene polymorphism in the pathogenesis of the diabetic nephropathy in T2DM subjects, it is suggested that SIRT1 gene polymorphism is a significant factor in the development and progression of DN.

In conclusion, the effects of SIRT1 gene polymorphisms on susceptibility to diabetic nephropathy might be mediated by differences in the metabolic state among individuals including glycemic control, obesity, and blood pressure also by difference in the ethnicity.

Therefore, the study of our interest suggests that SIRT1 may be a good candidate for diabetic nephropathy, although the association should be evaluated further in independent studies to obtain a precise conclusion.

It is recommended that further studies should be conducted on the association of SIRT1 gene polymorphism in the pathogenesis of diabetic nephropathy among different ethnicities, different genetic background, and large number of population for precise conclusion.

Studying the effect of SIRT1 combined with other genes as FOXO1 and other co-factors as P300 and screening more loci within SIRT1 gene should be taken in consideration.

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Disclosure

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