Role of SIRT-1 Gene in The Development of Diabetic Retinopathy

Dr. Laila H. Fawzy[1], Dr. Fatma Salah El-Din[2], Dr. Salwa I. Elshennawy[1], Dr. Marwa A. Fouly[2], Dr. Mai H. Salem[2]

[1]*Department of Clinical Pathology, Faculty of Medicine for Girls, Al-Azhar University,*
[2]*Research Institute of Ophthalmology, Scientific Research Academy*
Email: drmai.h.salem@gmail.com

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SIRT1, diabetic retinopathy; ELISA; gene expression.

ABSTRACT:
Background: Diabetes mellitus (DM) has had a consistent increase in global prevalence. A common microvascular disease associated with diabetes is diabetic retinopathy (DR). As a complex, progressive, as well as asymptomatic neurovascular consequence of DM, DR is the leading cause of vision impairment and blindness in working-age people. The presence of retinal neovascularization classifies diabetic retinopathy (DR) as proliferative, non-proliferative, or diabetic macular edema.
Aim of the Study: to evaluate the level of expression of mRNA sirt1- gene in cases of type 2 DM that have retinopathy or vulnerable to it.
Patients and Methods: In this research, 60 cases (15 patients with diabetic retinopathy, 15 diabetic cases without retinopathy (PDR), and 30 non-diabetic controls) were recruited. SIRT1 mRNA expression from the two groups was analyzed. Notably, patients with DR exhibited a decrease in the expression of SIRT1 mRNA. ELISA was also used to measure the levels of IL-17 expression in the serum. The results showed that the sera of people with DR had higher levels of IL-17 expression than the sera of healthy people.
Results: The findings of this research indicate that imbalanced IL-17 & SIRT1 expression levels may play a role in the etiology of DR, with SIRT1 potentially exerting a protective effect by suppressing IL-17 production.
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1. Introduction

Hyperglycemia is thought to be the main source of harmful functional, structural, and metabolic alterations in DR, which is a leading cause of adult acquired blindness [(1)].

Chronic hyperglycemia, nephropathy, hypertension, and dyslipidemia are all factors that are associated with or increase the risk of retinopathy. Intensive diabetes treatment, with the goal of achieving near-normoglycemia, has been shown to prevent and/or postpone the start and progression of DR, based on large prospective randomized studies [(2)].

Chronic uncontrolled hyperglycemia induces neuronal and vascular injury, which includes the formation of degenerative capillaries and the loss of ganglion cells. Additionally, it activates protein kinases and polyol pathways and increases oxidative stress. Nevertheless, the precise molecular mechanism that results in the development of DR remains unknown [(3)].

SIRT-1 is a class III histone deacetylase that modulates several physiological functions, such as inflammatory responses, apoptosis, as well as cell proliferation, through interactions with target proteins. It belongs to the silent information regulator-2 family [(4)].

The availability of cellular NAD determines the activity of SIRT-1, a protein that is primarily found in the nucleus. Sirt-1 is expressed all across the retina and, when it's upregulated, it protects against a number of eye illnesses, such as cataracts, optic neuritis, as well as retinal degeneration [(5)].

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SIRT-1 also regulates gene transcription through the modification of the acetylation status of the transcription factor or the regulation of epigenetic modifications at the transcriptional factor binding site of a gene [(7)].

The suppression of SIRT-1 is a contributing factor to the etiology of DR, as it results in hyperacetylation and the activation of nuclear transcription factor-kB (NF-kB). This transcriptional activation of mitochondria-damaging matrix metalloproteinase (MMP-9) is significant [(8)].

Regulation of oxidative stress prevents the reduction of SIRT-1 activity in retinal vasculature. In diabetes, the regulation of oxidative stress, together with NAD modulation, governs SIRT-1 through the management of posttranslational modifications and protein interactions [9].

2. AIM OF THE STUDY

The objective of this research is to evaluate the level of expression of mRNA SIRT-1 gene in cases of type 2 DM that have retinopathy or vulnerable to it.

3. SUBJECTS AND METHODS

This case-control trail was performed on sixty subjects, recruited from outpatient clinic at Research Institute of Ophthalmology (RIO) Hospital.

The subjects were classified into 2 groups: Group I: 30 type 2 diabetic patients, further divided into group I(A): 15 diabetic cases without retinopathy, group I(B): 15 DR cases, group II: 30 apparently healthy subjects as a control.

Inclusion criteria:

- Cases: Type 2 diabetic patients with fundus changes compatible with retinopathy diagnosis.
- Diabetic controls: Patients suffering from Type 2 DM without retinopathy.
- Apparently healthy controls: Healthy control subjects.

Exclusion criteria:

- Type 1 DM.
- Individuals with diseases other than T2DM, involving CNS diseases such as stroke, tumor, Parkinson's disease, history of brain trauma, seizure disorder, acute and chronic infections, renal diseases & failure.
- History of glaucoma or epilepsy.
- Autoimmune disease. Or eating disorder
- Eye conditions that obscure retinal lesions, such as glaucoma, dense cataracts, or severe visual impairment, and prior vitreal surgery
- Any healthy control subject with high fasting blood glucose.

Methodology:

1. Clinical and ophthalmological evaluations:

All participants of this study were diagnosed and graded by an expert ophthalmologist at the Medical Retina Clinics of RIO hospital and subjected to the following: full clinical history: duration of diabetes, medications, history of hypertension, hyperlipidemia, smoking, retinal surgery, autoimmune diseases, CNS & cardiovascular disease. Estimation of BMI; weight and height of all subjects were measured. Full ophthalmic examination through dilated pupils: Visual acuity, slit-lamp and fundus examination, fundus fluorescein angiography (FFA) in addition optical coherence tomography (OCT) was needed in some patients.

2. Laboratory investigations:

Fasting blood sugar (FBS), complete lipid profile (triglycerides, Total cholesterol, LDLC, HDL-C), glycated Hemoglobin (HbA1c) blood Level, microalbumin in urine (MAU), measuring the expressions of messenger RNA of SIRT 1gene and estimation of serum IL-17.

3. Laboratory methodology:

a. Fasting blood glucose: By Erba Mannheim XL Sys Pack (GLU 440 kit) Cat.No. XSYS0012, using (automated autoanalyzer), *1®Erba XL instrument.

b. Lipid profile:

I. Triglycerides

The automated autoanalyzer *®Erba XL instrument was used to conduct an enzymatic colorimetric assay using lipoprotein lipase enzyme. Utilizing Erba's products. Lipase catalyzes the enzymatic hydrolysis of triglycerides to produce glycerol and free acids. The glycerol is phosphorylated by glycerol kinase (GK) in the presence of adenosine triphosphate (ATP) to generate glycerol 3-phosphate and adenosine diphosphate (ADP). Glycerol phosphate oxidase generates hydrogen peroxide (H2O2) by oxidizing glycerol 3-phosphate to dihydroxy-acetone phosphate. A red dye is produced by the reaction of H2O2 with 4- aminoantipyrine (4AAP) and 4-chlorophenol in a Trinder type color reaction catalyzed by peroxidase. A direct proportional relationship exists between the absorbance of this dye and the concentration of the dye in the sample.

Total cholesterol

The cholesterol esterase method was employed to measure serum total cholesterol using an enzymatic colorimetric assay on an automated autoanalyzer, the *®Erba XL instrument. Utilizing Erba's products. The enzymatic hydrolysis of cholesterol esters is catalyzed by cholesterol esterase, which results in the production of cholesterol as well as fatty acids that have been freed. Free cholesterol, including the cholesterol that was initially present, is converted into cholest-4-en-3-one and hydrogen peroxide by the enzyme known as cholesterol oxidase. By combining H2O2 and 4-aminoantipyrine, it is possible to determine the concentration of a chromophore, also known as Quinoneimine dye, at a wavelength of 505 nm.

II. HDL cholesterol

Determination of HDL-C was done by enzymatic colorimetric assay after the precipitation by magnesium chloride / dextran sulfate reagent by (automated autoanalyzer) *®Erba XL instrument, with kits supplied by Erba. HDL-C levels were determined directly utilizing a two-reagent format, eliminating the necessity for sample pretreatment or specialized centrifugation procedures. The initial reaction involved the formation of water-soluble complexes among dextran sulfate and chylomicrons, VLDL, and LDL in the presence of magnesium sulfate. The cholesterol oxidase and cholesterol esterase that reacted with HDL-C were polyethylene glycol (PEG)-modified, and these complexes were resistant to them. The HDL-C was oxidized to Δ4-cholestenone and H2O2 in the presence of O2. In the presence of peroxidase, the H2O2 that was generated subsequently reacted with sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HSDA) as well as four aminoantipyrine to generate a colored dye. The bichromatic (600/700 nm) endpoint technique was employed to ascertain the presence of this dye. The dye's color intensity was directly proportional to the concentration of serum HDL-C.

III. LDL cholesterol

- • The Friedewald Equation (1972) was employed to calculate LDL-C.

c. Glycated hemoglobin (HbA1c):

Glycated hemoglobin measurement was based on particle enhanced immunoturbidimetric method, on (automated autoanalyzer) *®Erba XL instrument, with kits supplied by Erba. There is no need to assess total hemoglobin in order to determine HbA1c. We used the Hemolyzing solution (R3) to completely lyse the samples. Hemolyzed hemoglobin and hemoglobin A1c attach to R1 (buffer and latex) particles with identical affinities. Both chemicals' relative concentrations in the blood determine the amount of binding. R2a, a mouse anti-human HbA1c monoclonal antibody, is capable of binding to HbA1c that is attached to particles. Agglutination takes place as a result of the interaction between the goat anti-mouse IgG polyclonal antibody (R2b) and the monoclonal mouse anti-human HbA1c antibody. There is a direct correlation between the amount of hemoglobin A1c that is bound to particles and the absorbance measurement, which is proportional to the HbA1c concentration of the sample [(10)].

d. Microalbumin in urine (MAU)

It is the MAU antigen-antibody reaction that serves as the foundation for this test. The kit that was provided by 2® Spectrum-diagnostics was utilized in conjunction with a particle-enhanced turbidimetric immunoassay that was performed on an automated autoanalyzer (Erba XL) of the equipment. In order to create a reference curve, serial dilutions of the standard in saline at a ratio of 1:2 were performed (at least four points). Saline was used as zero point. Δ absorbance of the sample and each standard was determined as following: Δ

absorbance of sample = (A2 - A1) sample Δ absorbance of each calibrator = (A2 - A1) for each standard The calibration curve was plotted and the results were obtained [11].

e. ELISA quantitative detection of human interleukin 17 (IL-17) in serum:

In order to produce a standard curve, the average OD of each standard was plotted along the vertical (Y) axis against the concentration down the horizontal (X) axis. The nodes on the graph were then used to create a curve that as the best fit for the data.

The most effective method for performing these calculations is to utilize computer-based curve fitting software. Regression analysis can be employed to determine the optimal fit line.

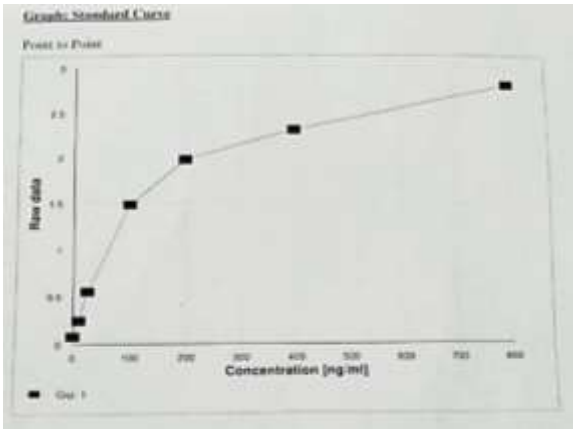

Fig. 1: Standard curve of ELISA.

f. Measuring the expression of messenger RNA of SIRT1 gene by quantitative real time PCR (q-PCR).

Fig. 2: Steps of RNA isolation

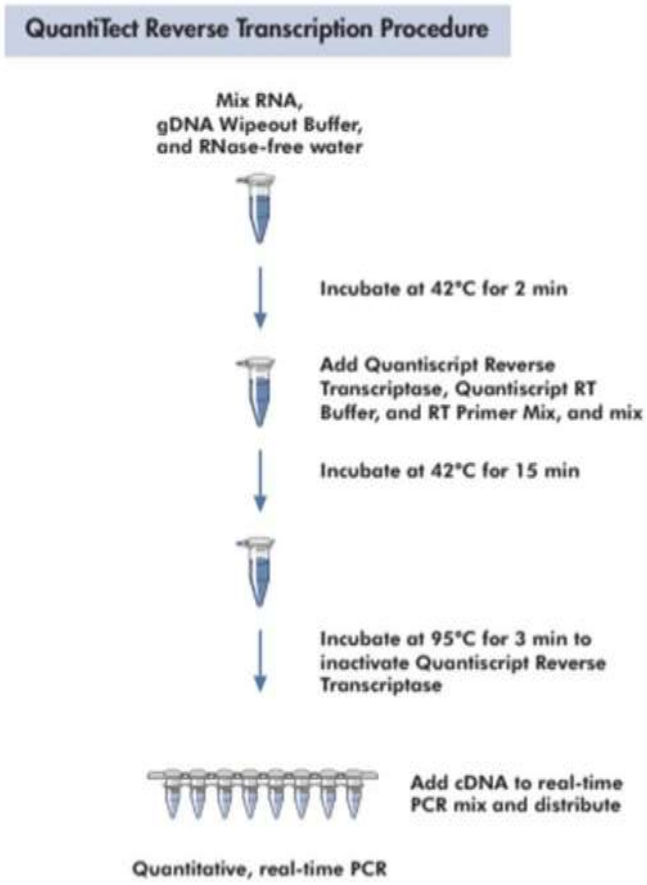

Fig. 3: Reverse transcription procedure

Real-time qPCR utilizing SYBR Green I:

The amplification and analysis of real-time qPCR were carried out with the assistance of an Applied Biosystems apparatus equipped with software version 3.1 (StepOne Plus™, USA). Using the primer sets, the quantitative polymerase chain reaction (qPCR) assay was optimized at the annealing temperature. The sequence of primers is shown in the table below.

Table 1: The primer sequence of the examined gene:

	Primer sequence
Sirt-1	Forward primer: 5'- TGTGGTAGAGCTTGCATTGATCTT-3′ Reverse primer: 5′- GGCCTGTTGCTCTCCTCAT -3′
GADPH	Forward primer: 5'- GCACCGTCAAGGCTGAGAA 3′ Reverse primer: 5′- TGGTGAAGACGCCAGTGGA -3′

Preparation of the reaction master mix for Q-PCR:

Volumes of the following reagents were added to each sample.

Table 2: PCR reaction mix component:

PCR reaction mix component	Volume
Forward Primer	1 µl
Reverse Primer	1µl
Syber green mix	12.5 µl
cDNA template	5 µl

RNAse free water	5.5 µl
Total volume	25 µl

Calculation of Relative Quantification (RQ) (relative expression)

The relative quantitation was determined by the following equation in accordance with the Applied Bio System software:

- Δ Ct = Ct gene test – Ct endogenous control
- ΔΔCt = ΔCt sample – ΔCt control

Statistical Analysis

Basic clinical examinations, laboratory investigations, and outcome assessments have all been coded, recorded, and analyzed using Microsoft Excel throughout history. We next imported the data into SPSS version 20.0, the Statistical Package for the Social Sciences, to do the analysis. Qualitative data is represented as a numerical number beside a percentage, while quantitative data is generally expressed as the mean with the addition or subtraction of the standard deviation. We employed the Chi-square test (X^2) to analyze the differences and associations of the qualitative variable. The subsequent tests were employed to ascertain the significance of the differences. The quantitative independent groups exhibited a difference of less than 0.32, as indicated by the t-test, Mann-Whitney U test, Spearman's correlation, and the P -value. The P value threshold was established at below 0.05 for significant results and below 0.001 for very significant results.

4. RESULTS

Table 3: Basic demographic data distribution between examined groups including; age, weight, Hight, BMI, sex and smoking.

			Control Group N=30	Diabetic Group N=30	t	p
Age			58.90±6.51	58.23±7.37	0.371	<0.712
Weight/ KG			87.50±16.01	90.96±19.58	0.750	<0.456
Height/ CM			168.43±14.32	170.0±13.48	0.436	<0.664
BMI			30.84±4.40	31.51±6.42	0.467	<0.642
Sex	Female	N	16	16		
		%	53.3%	53.3%		
	Male	N	14	14	0.0	<1.0
		%	46.7%	46.7%		
Smoker	No	N	30	26		
		%	100.0%	86.7%		
	Yes	N	0	4	2.79	<0.11
		%	0.0%	13.3%		
		%	10.0%	26.7%		
Total		N	30	30		
		%	100.0%	100.0%		

Age was distributed as 58.90±6.51 and 58.23±7.37 with no significant variation among groups also there was no significant distinction regard Weight or Height or BMI regard sex distribution groups were matched and there was no significant regard smoking distribution.

Table 4: LAB distribution between studied groups including total cholesterol, triglycerides, HDL, LDL, FBS, HbA1C and MAU:

	Control Group N=30	Diabetic Group N=30	t/ Mann Whitney	P
Total cholesterol mg/dl	243.40±66.10	267.76±55.40	1.547	<0.127
Triglycerides mg/dl	104.13±33.52	128.80±41.79	1.989	<0.051
HDL mg/dl	43.06±13.53	37.10±10.08	1.847	<0.070
LDL mg/dl	183.83±57.97	208.83±62.89	1.895	<0.068
FBS mg/dl	88.13±12.46	180.93±60.01	5.851	<0.00**

HbA1c	4.0±0.37	8.53±1.28	18.551	<0.00**
MAU mg/l	15.50±5.23	23.66±7.85	3.482	<0.001**

FBS and HA1c were significantly higher amongst diabetic group also MAU was significantly higher amongst diabetic group.

Table 5: SIRT1 Gene expression and IL17 level distribution among examined groups:

	Control Group N=30	Diabetic Group N=30	Mann Whitney	P
PCR_CT	0.78±0.31	0.67±0.24	0.179	<0.813
IL17 ng/ml	5.74±1.95	6.60±1.95	1.812	<0.078

Insignificant decrease in SIRT1 gene expression amongst diabetic group in contrast to control group. Insignificant increase of IL17 level among diabetic group contrasted with control group.

Table 6: Basic demographic data distribution among diabetic group between group 1(A) and group 1(B): including; age, weight, Hight, BMI, sex, HPN and smoking.

			Diabetic without retinopathy Group1(A) (N=15)	Diabetic with retinopathy Group1(B) (N=15)	t	P
Age			57.40±8.02	59.06±6.83	0.612	<0.545
Weight/KG			85.46±18.24	96.46±19.92	1.577	<0.126
Height/CM			167.06±9.75	172.93±16.22	1.200	<0.240
BMI			30.52±5.48	32.49±7.30	0.835	<0.411
Diabetes duration			12.06±4.02	16.20±5.25	1.597	<0.122
Sex	Female	N	8	8	0.0	<1.0
		%	53.3%	53.3%		
	Male	N	7	7		
		%	46.7%	46.7%		
Smoker	No	N	11	15	2.59	<0.10
		%	73.3%	100.0%		
	Yes	N	4	0		
		%	26.7%	0.0%		
HPN	No	N	10	12	0.68	<0.40
		%	66.7%	80.0%		
	Yes	N	5	3		
		%	33.3%	20.0%		
Total		N	15	15		
		%	100.0%	100.0%		

No significant variation amongst groups regard age, weight, height or BMI and regard sex distribution groups were matched and also there was no significant distinction regard smoking or HTN distribution between studied groups.

Table 7: LAB data distribution among diabetic group between group 1(A) and 1(B) including total cholesterol, triglycerides, HDL, LDL, FBS, HbA1c and MAU:

	Diabetic without retinopathy Group1(A) (N=15)	Diabetic with retinopathy Group1(B) (N=15)	t	P
Total cholesterol mg/dl	241.80±69.49	245.0±64.93	0.130	<0.897
Triglycerides mg/dl	118.67±32.0	138.93±42.63	1.074	<0.292
HDL mg/dl	43.40±11.37	42.73±13.58	0.123	<0.903
LDL mg/dl	176.06±59.63	181.60±60.36	0.219	<0.828
FBS mg/dl	187.60±64.5	174.26±58.63	0.419	<0.679
HbA1c	8.02±1.23	9.04±1.15	2.355	<0.026*
MAU mg/l	19.40±6.87	27.93±9.14	2.318	<0.028*

HbA1c and MAU were significantly higher among group 1(B).

Table 8: LAB data distribution among diabetic group between group1(A) and group 1(B) including PCR/CT and IL17 level:

	Diabetic without retinopathy Group1(A) (N=15)	Diabetic with retinopathy Group1(B) (N=15)	Mann Whitney	P
PCR/CT	0.84±0.32	0.43±0.24	2.738	<0.011*
IL17 ng/ml	5.75±2.08	7.52±1.71	2.782	<0.010*

SIRT1 Gene expression was significantly lower among group1(B) compared to group 1(A) but IL17 was significantly higher.

Table 9: Correlation between gene expression and other parameters:

		PCR_CT
Age	r	-.037-
	P	.845
BMI	r	.059
	P	.759
Diabetes duration	r	-.080-
	P	.674
Total cholesterol mg/dl	r	.050
	P	.794
Triglycerides mg/dl	r	.300
	P	.107
HDL mg/dl	r	.093
	P	.626
LDL mg/dl	r	-.012-
	P	.948
FBS mg/dl	r	.224
	P	.233
HbA1c	r	-.329-
	P	.076
IL17 ng/ml	r	-.390-*
	P	.033
MAU mg/l	r	-.132-
	P	.486

No significant correlation founded except significant negative correlation between PCR/CT and IL17.

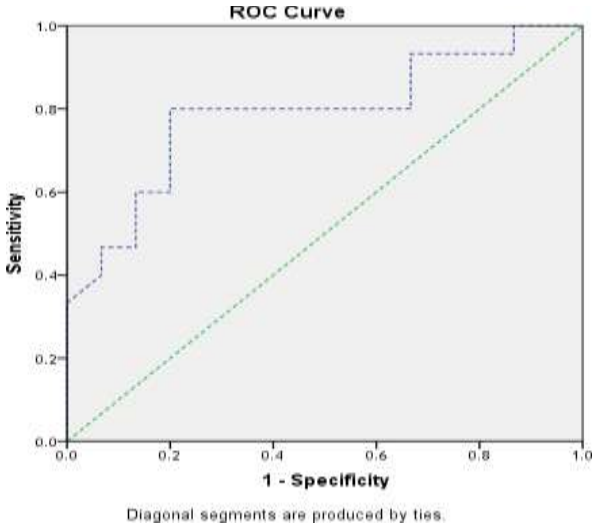

Fig. 4: ROC curve for detection of PCR/CT cutoff regard retinopathy.

Table 10: AUC, cutoff & validity of PCR/CT:

Area	Cutoff	P	95% Confidence Interval		Sensitivity	Specificity
			Lower Bound	Upper Bound		
0.789	<0.59	0.007*	0.620	0.957	80.0%	80.0%

Significant AUC (cutoff <0.59) with 80.0 percent sensitivity as well as 80.0 percent specificity.

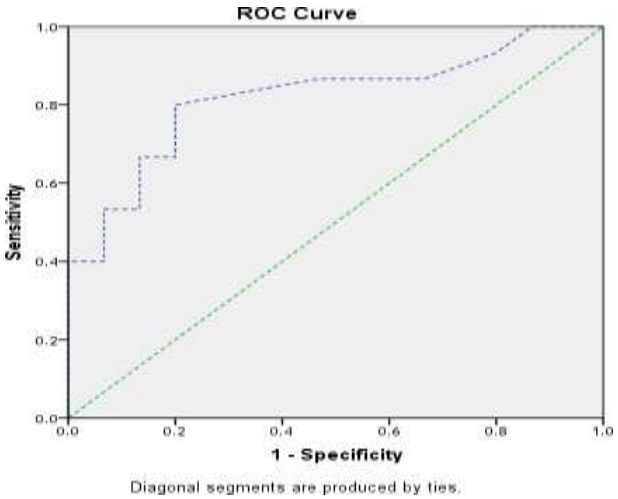

Fig. 5: ROC curve for detection of IL17 cutoff regard retinopathy.

Table 11: AUC, cutoff and validity of IL17:

Area	Cutoff	P	95% Confidence Interval		Sensitivity	Specificity
			Lower Bound	Upper Bound		
0.820	>6.6	0.003*	0.663	0.977	80.0%	78.0%

Significant AUC with cutoff >6.6 with sensitivity & specificity 80.0% and 78.0% respectively.

5. DISCUSSION

DM is a chronic metabolic disease associated with abnormal blood glucose levels because of either insufficient insulin production or lack of cell sensitivity to its action. It is considered as a chronic disease that can affect carbohydrate, protein, and fat metabolism [(12)]. Ageing and rapid lifestyle changes induce the worldwide and fast-growing epidemic of diabetes which is expected to be the chief cause of death in the coming decades [(13)].

Diabetic retinopathy is the most prevalent neurovascular complication of diabetes mellitus. The inflammatory effects of hyperglycemia and dyslipidemia compromise the arteries that deliver oxygen and nutrients to the retinal cells. The compromised vessels commence leaking, resulting in retinal swelling and hypoxia. The retina responds to this degradation by secreting growth factors, such as vascular endothelial growth factor (VEGF), which facilitates the formation of new blood vessels. The newly created vessels are highly delicate; they are prone to bleeding and may get obstructed, leading to exacerbation of ischemia [(14)].

Silent Information Regulator 2 (SIR2) is a member of the histone deacetylase family. It is recognized for its anti-inflammatory and oxidation-resistant properties. SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, and SIRT7 are the seven proteins that share characteristics with SIR2 in terms of their structural similarity. It has been determined that SIRT1 plays a significant part in the establishment of DR [(15)].

Cell proliferation, apoptosis, and inflammatory responses are just a few of the numerous physiological processes that might be improved by the class III histone deacetylase SIRT 1 [(4)]. SIRT1 is expressed in the brain, liver, heart, pancreas, spleen, skeletal muscle & adipose tissues. SIRT1 is widely expressed in the retina, and its overexpression confers protection against several ocular disorders, including retinal degeneration, cataracts, and optic neuritis [(16)]. SIRT1 is located in both the nucleus and cytoplasm, predominantly expressed in the nucleus, and its activity is contingent upon the availability of cellular NAD. SIRT1 has been shown to safeguard cells against oxidative stress in several disease conditions related to aging, neurodegeneration, metabolic disorders, cancer & cardiovascular illnesses [(17)]. SIRT1 expression has been demonstrated to be

diminished in the retina as well as its capillary cells in diabetes [6]. SIRT1 controls gene transcription through editing the transcription factor's acetylation status or by influencing epigenetic changes at the gene's transcription factor binding site [7].

The case control research was conducted at the out patients' clinics of research institute of ophthalmology. Thirty patients of T2DM (Group I) were compared to 30 non diabetic apparently healthy controls (Group II). Diabetic patients have been subdivided into two further groups (15 diabetic patients without retinopathy (Group IA), 15 diabetic cases with retinopathy (Group I B). The characteristics of the patients and controls were all well matched regarding age, sex, weight, height & BMI. SIRT1 mRNA expression levels in the whole blood were analyzed using RT-PCR. Our results revealed SIRT1 gene expression was insignificantly lower among (group I) compared to (Group II). However, data distribution among the subdivided diabetic (Group IA) and (Group I B), showed that SIRT1 gene expression was significantly lower among the (Group I B), diabetic patients who have developed DR.

The downregulation of SIRT1 leads to hyperacetylation of numerous regulatory proteins, involving transcription factors linked to oxidative stress & apoptosis. In accordance with our findings Tu et al. [18] discovered that In contrast to normal mice, SIRT1 expression was diminished in DR mice. The development of DR was alleviated by the deacetylation of SIRT1 target genes Foxo1 and NF-κB, which inhibited oxidative stress and inflammation. Same results were reported by Liu et al. [19]. Chinese study that showed that the expression levels of SIRT1 mRNA as well as protein were reduced in the PBMCs of individuals with DR.

Song et al. [20] showed that in T2DM subjects, SIRT1 expression was suppressed in cells of diabetic patients. Al-khaldi and Sultan [21] found that the level of SIRT1 mRNA expression was found to be significantly lower in the individuals who were diagnosed with type 2 diabetes contrasted with the control group. These results approved the protective role of SIRT1 against diabetic vascular complication through counteracting oxidative stress [22]. Differences in the number of cases selected in each research, the environment, nutrition, ethnicity, or the patients' ethnicity could all contribute to the variability of the results. In contrast with the present hypothesis Maloney et al. [23] found that, SIRT1 expression was upregulated. Further research is needed to determine the exact mechanism at work; however, it might be a result of a defensive feedback system in the retina.

In our study, serum IL-17 levels were insignificantly increased in diabetic individuals, as compared with control subjects. IL17 was significantly higher in (Group I B) contrasted with (Group IA) which was in accordance with previous findings that were showed by Wang et al. [24] who establish that IL-17A is actively involved in DR pathophysiology as it promotes retinal ganglion cells (RGC) death through mediation of by retinal muller cells (RMC). Lee et al. [25] have identified SIRT1 as a regulator of inflammatory reactions, namely by inhibiting IL17 production [26]. In accordance with our findings, a study conducted on Chinese type 2 diabetic patients by Liu et al. [19] demonstrated that Individuals with DR exhibited elevated levels of IL-17 in their PBMCs, while SIRT1 mRNA and protein expression levels were reduced in their PBMCs. The results suggested a negative correlation between IL-17 and SIRT1, which could implicate SIRT1 in the etiology of DR. Consequently, SIRT1 may have protective effects in DR.

Our study demonstrated a statistically significant elevation of HbA1c in diabetes cohorts relative to the healthy control group, as well as a statistically significant rise in HbA1c in the DR group in contrast to the diabetic group without retinopathy. HbA1c is regarded as a crucial biomarker of diabetes management, reflecting hyperglycemia, the primary risk factor in the development and progression of DR [27]. Our study indicates that the DR group exhibits elevated HbA1c levels contrasted with the diabetic group without retinopathy, confirming that the DR group is less managed and consequently at a greater risk for diabetes-related problems, namely DR.

In our study, TC of diabetic patients insignificantly increased compared to apparently healthy control group ($P<0.127$). TC showed insignificant increasing in Group IB contrasted with Group IA ($p<0.897$). LDL-c insignificantly raised $P<0.068$ in diabetic group when compared to control group, and insignificantly increased comparing Group IB and Group IA ($p<0.828$). Individuals who have type 2 diabetes experience a decrease of normal insulin sensitivity, which leads to compensatory hyperinsulinemia. This can be ascribed to the disorder. The activation of lipase in adipose tissue, which leads to an increase in free fatty acids (FFAs), is associated with insulin resistance being present. Free fatty acids circulate in the bloodstream, are absorbed by the liver, and undergo esterification to form very low-density lipoproteins, which are subsequently released into the

bloodstream. In the blood arteries, VLDL is transformed into IDL and subsequently into LDL [28]. HDL-c insignificantly decreased in diabetic cases when contrasted with healthy individuals (P<0.07) and in Group IB when compared to Group IA (p<0.903) Song et al. [20] have revealed that the expression of hepatic lipase is elevated in diabetics, which affects HDL-c. This results in smaller HDL particles that are more promptly catabolized by the kidney, resulting in a decrease in plasma HDL-c. In terms of TG, diabetics exhibited a statistically insignificant increase in comparison to the control group and group IB in contrast to group IA. Rutledge et al. [29] suggested that Enhanced hepatic secretion of VLDL and delayed clearance of triglyceride-rich lipoproteins may be the mechanism that underlies hypertriglyceridemia. This is primarily attributable to elevated levels of substrates for triglyceride production, specifically increased levels of free fatty acids and glucose. Insignificant results may be attributed to ow number of studied cases.

In our study MAU level is significantly increased in diabetic group contrasted with control and significantly increased in group IB in relation to group IA. In agreement with our results Ajoy-Mohan et al. [30] and Sobngwi et al. [31] revealed that Microalbuminuria is strong predictor for DR, in type 2 diabetics and could be considered as a sensitive marker of early DR.

6. CONCLUSION

Our results suggested that in DR SIRT1 gene expression is downregulated and IL17 serum level is increased. Therefore, SIRT1 may have protective effects in the development of DR.

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