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CORRELATION BETWEEN GLYCAEMIC CONDITIONS WITH SIRT-1 GENE EXPRESSION LEVELS IN THE PROGRESSION OF DIABETIC RETINOPATHY

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Abstract

***Introduction*:** Diabetic retinopathy (DR), a microvascular disorder, is commonly linked to diabetes. Diabetes mellitus' complex, progressive, and asymptomatic neurovascular effects make DR the leading cause of blindness and visual impairment in people of working age. Retinal neovascularisation determines whether DR is proliferative, non-proliferative, or diabetic macular oedema.

Aim: To evaluate the levels of SIRT-gene mRNA expression and how they relate to glycaemic status in patients with diabetic retinopathy.

Methods: The 60 cases in this study included 30 patients with diabetic retinopathy, 30 diabetic cases without retinopathy, and 30 non-diabetic controls. SIRT1 mRNA expression and basic laboratory measures were recorded.

***Results*:** It was demonstrated that DR patients have down-regulated SIRT1 mRNA expression. The results of the study suggest that DR results from an imbalance between SIRT1 and IL-17 expression levels, with SIRT1 perhaps offering protection by preventing the production of IL-17. The DR cases had significantly lower levels of SIRT1 gene mRNA expression than the controls. The association between SIRT1 mRNA expression and four putative functional SNPs (rs12778366, rs3758391, rs2273773, and rs4746720) was examined. The findings showed that the allelic genes of rs3758391 showed substantial changes in SIRT1 mRNA expression in the healthy subjects ($p<0.01$). Compared to the controls (0.91±0.75 fold), SIRT1 mRNA expression levels were lower in the RD (0.61 ± 0.29 fold). SIRT1 gene expression decreases when HbA1c rises, indicating a negative connection between SIRT1 expression and HbA1c. This suggests that SIRT1 plays a protective function in preventing diabetic retinopathy ($r = -0.245$, $p = 0.004$). When comparing the Diabetes with Retinopathy Group to the Diabetes without Retinopathy Group1, SIRT1 Gene Expression was considerably lower, but IL17 was significantly greater (0.62±0.30 vs. 0.60±0.27, $p=0.012$).

Conclusion: In conclusion, the present data provide credence to the idea that the rs3758391 SNP affects mRNA expression in healthy people and that the SIRT1 gene guards against DR. In connection with the pathophysiology of DR, they also clarified the processes controlling the genetic regulation of the SIRT1 gene.

Keywords: Diabetic Retinopathy, SIRT-1 gene, neovascularization, microvascular disease. Cite This Article: SHAIK, Mahaboob Vali et al. CORRELATION BETWEEN GLYCAEMIC CONDITIONS WITH SIRT-1 GENE EXPRESSION LEVELS IN THE PROGRESSION OF DIABETIC RETINOPATHY. International Journal of Retina, [S.l.], v. 8, n. 2, p. 1, sep. 2025. ISSN 2614-8536. Available at: <https://www.ijretina.com/index.php/ijretina/article/view/317>. Date accessed: 30 sep. 2025. doi: https://doi.org/10.35479/ijretina.2025.vol008.iss002.317..

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INTRODUCTION

Persistent, uncontrolled hyperglycemia causes vascular and neuronal damage, including the death of ganglion cells and the formation of degenerative capillaries. Diabetic Retinopathy (DR) is still the most common cause of acquired blindness in people of working age, and high blood glucose is believed to be the main cause of detrimental structural, functional, and metabolic changes. The exact chemical mechanism that causes diabetic retinopathy is yet unknown. The entire retina expresses the nuclear protein i.e. Silent mating type information regulator 2 homolog 1 (SIRT1). The upregulation of SIRT1 prevents a number of visual disorders, including cataracts, optic neuritis, and retinal degeneration.[1] A study found that diabetes lowers Sirt1 activity and expression in the retina's capillary cells.[2]

SIRT1 is an atheroprotective factor in vascular biology. In certain populations, genetic variations in SIRT1 are associated with coronary artery calcification and type 2 diabetes. SIRT1 belongs to the silent information regulator-2 family.[3,4,5]

SIRT-1 suppression contributes to the aetiology of DR because it increases nuclear transcription factor-kB (NF-kB) and promotes hyperacetylation.[6,7] Until oxidative stress is managed, SIRT-1 activity in the retinal vasculature cannot be reduced.[8,9,10,11] The mRNA expression of the SIRT1 gene is associated with single nucleotide polymorphisms (SNPs) in metabolic disorders. SNPs in the SIRT1 gene may alter the messenger Ribonucleic Acid (mRNA) expression by altering the ability of transcription factors or mRNAs to bind. To determine the role of SIRT1 in DR, we compared the expression levels of SIRT1 in peripheral blood mononuclear cells (PBMCs) between DR patients and controls using real-time PCR replication. Four putative functional SNPs in SRIT1 were assessed to see if they affect the levels of mRNA expression of the SIRT1 gene. Till there is an unresolved clarity regarding the association between gene expression levels of SIRT-1 and the severity of diabetic retinopathy. Current study formulated to evaluate the level of expression of mRNA of SIRT-1 gene in patients with and without retinopathy. With this study it may give some inclusions which may fulfill the role of SIRT-1 and it's mechanism in the development of diabetic retinopathy.

METHODS

Study Design and Setting: This case-control study involved 60 participants who were enrolled in the Ophthalmology Outcomes Department. The study conformed with requirements set in the Declaration of Helsinki. Study protocol approved by Institutional ethics committee, NIMS, Vijayawada. (IEC/2024/OPH/RC-03). Informed consent forms obtained from study participants or their attenders. Two groups of participants were formed. In group I, 30 participants with diabetic retinopathy and 30 diabetics without retinopathy. Whereas in group II, 30 healthy control participants were included.

Patient Selection and Sampling: Patients with type 2 diabetes with diagnosis of retinopathy (n=30) were included as Cases. While individuals with Type 2 diabetes without retinopathy (n=30) were included as Diabetic controls. Whereas study participants without diabetes and good health (n=30) were included as Healthy controls. Patients excluded those with type 1 diabetes mellitus, individuals with stroke, tumour, Parkinson's disease, brain trauma history, seizure disorders, acute and chronic infections, and renal illnesses and failure or those affects central nervous system, history of epilepsy (or) glaucoma, immune system disorders, glaucoma, dense cataracts, or visual impairment which mask retinal abnormalities, and any previous vitreal surgery.

Data Collection: After obtaining written informed consent, data collected such as full medical history, duration of diabetes, medications, history of

hyperlipidaemia, high blood pressure, smoking, retinal surgery, autoimmune diseases, and illnesses of the central nervous system and cardiovascular system.

Ophthalmic examination: Optical coherence tomography (OCT), Fundus fluorescein angiography (FFA), Slit-lamp & fundus examination, and Visual acuity. Laboratory investigations undergone such as Fasting blood sugar (FBS), lipid profile, glycated Hemoglobin (HbA1c) Level, urine microalbumin levels, expression levels of messenger RNA of SIRT 1gene & serum IL-17 levels.

DNA Extraction & genotyping: A DNA extraction kit was used to extract genomic DNA from blood leukocytes in accordance with a standard procedure. The SNPs were genotyped using the Taqman genotyping method (Real-time PCR equipment, Applied Biosystems).

Thermocycler's settings such as 10 minutes at 95 °C, 40 cycles of 15 seconds at 95 °C, and one minute at 60 °C. 2% of the samples were selected at random & genotyped in duplicate, whereas > 98 % of the samples were successfully genotyped. The samples were separated from one another by less than 1%.

Primer & probe sequences of variants at SIRT1 locus:

rs3758391-F : 5'- TGCCATAACAAACACTGGCTCTA -3'
rs3758391-R : 5'- GCACACTGTGACTCCATATCTAATCTTA -3'
rs3758391-P-T : 5'- FAM-ATCTACCA***T***GGGTTATAT-MGB -3'
rs3758391-P-C : 5'- HEX-ATCTACCA***C***GGGTTAT-MGB -3'
rs12778366-F : 5'- GCTTCTAGGACTGGAGATGATTACTTTC -3'
rs12778366-R : 5'- TCCTATCTACATCCAAAAGTCTTATTTCA -3'
rs12778366-P-T : 5'- FAM-AAATGAA***T***AGTGGTGACCAG-MGB -3'
rs12778366-P-C : 5'- HEX-AAATGAA***C***AGTGGTGACC-MGB -3'
rs4746720-F : 5'- CACTTTTCTTTGTAACATTGAATGGTTT -3'
rs4746720-R : 5'- TGCCAGTGTTTAAAAATAATTGTGTT -3'
rs4746720-P-C: 5'- FAM-CGCTAAACTT***C***TGATTC-MGB -3'
rs4746720-P-T : 5'- HEX-CGCTAAACTT***T***TGATTC-MGB -3'
rs2273773-F : 5'- TCCAGCCATCTCTCTGTCACAA -3'
rs2273773-R : 5'- GCGTGTCTATGTTCTGGGTATAGTTG -3'
rs2273773-P-T : 5'- FAM-TTCATAGCC***T***TGTCAGAT-MGB -3'
rs2273773-P-C : 5'- HEX-TTCATAGCC***C***TGTCAGA-MGB -3'

RNA extraction: Peripheral blood was obtained from all study participants, and PBMCs were separated using Lymphocyte Separation Medium in accordance to the manufacturer's instructions. Total RNA was extracted from the PBMCs using the TRIzol reagent in accordance with the manufacturer's instructions. The amount of RNA was measured using a Thermo Scientific NanoDrop2000 spectrophotometer. All of the RNA samples were stored at 80°C before cDNA synthesis, hybridisation, and labelling.

cDNA Synthesis and Real-Time PCR Detection: Total RNA (~1 µg) was converted to cDNA using reverse transcription. SIRT1 and IL-6 real-time PCR were carried out in triplicate using SYBR Green. Gene expression levels were assessed using a CFX96 RT-PCR detection system (Bio-Rad), per the manufacturer's recommendations. After combining RNA with DNA wipeout buffer and RNAse-free water, the mixture was incubated at 42^0C for two minutes. Reverse transcriptase, RT primer mix, and RT buffer were then added, and the mixture was incubated at 42^0C for 15 minutes. The reverse transcriptase was then incubated at 95^0 C for three minutes to inactivate it. Include cDNA in real-time PCR and distribute it for quantitative real-time PCR. The relative mRNA expression levels were normalised to those of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and then analysed using the ΔΔCt technique.

Calculation of Relative Quantification (RQ) (relative expression):

The relative quantitation is determined by the formula (Applied Bio System):

Δ Ct = Ct gene test – Ct endogenous control

ΔΔCt = ΔCt sample – ΔCt control

Primers used for gene expression analysis as below.

SIRT-1: Forward primer:5′- TGTGGTAGAGCTTGCATTGATCTT-3′

SIRT-1: Reverse primer: 5′- GGCCTGTTGCTCTCCTCAT -3′

GADPH: Forward primer: 5′- GCACCGTCAAGGCTGAGAA 3′

GADPH: Reverse primer: 5′- TGGTGAAGACGCCAGTGGA -3′

The components of master mix for q-PCR were 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.

The amplification program consists: one cycle at 50 ℃ for 2 minutes, followed by another cycle at 95 ℃ for 2 minutes, followed by 40 cycles at 95 ℃ for 15 seconds & 60 ℃ for one minute.

Statistical analysis: Results of fundamental research have been evaluated using the Statistical Package for the Social Sciences (SPSS) version 20.0. Percentages are used to convey qualitative data, while the mean and standard deviation are used to convey quantitative data. The chi-square test was used to analyse the associations and differences of the qualitative variable. The t-test, Mann-Whitney U test, and Spearman's correlation all indicated that the quantitative independent groups differed by less than 0.32. The P value criterion was set at less than 0.05 for significant results and less than 0.001 for highly significant results.

RESULTS

Table 1: Basic demographic data distribution between the groups

			Control, n=30	Diabetic cases, n=60 (diabetes without retinopathy + diabetes with retinopathy)	P value
Age			57.8 ± 8.4	56.2±7.5	0.65
Weight (kilograms)			75.50 ± 16.01	72.6±20.6	0.45
Height(centimeters)			165.5 ± 15.32	167.0±14.5	0.72
BMI			25.6 ± 4.40	26.51±6.42	0.64
Sex	Female	n	17	32	0.85
		%	56.66%	53.3%	
	Male	n	13	28	
		%	43.34%	46.7%	
Total		n	30	60	
		%	100.0%	100.0%	

BMI: body mass index, p value significant at <0.05.

There was no discernible variation in the age distribution between the groups. When the sex groups were matched, there was no significant change in the distribution of height, weight, or BMI.

Table 2: Laboratory variables in two groups

	Control N=30	Diabetic cases, n=60 (diabetes without retinopathy + diabetes with retinopathy)	P value
Total cholesterol (mg/dl)	233.40±66.10	255.76±55.40	0.127
Triglycerides (mg/dl)	101.13±33.52	136.80±41.79	0.055
HDL cholesterol (mg/dl)	44.2±13.53	36.10±10.5	0.069
LDL cholesterol (mg/dl)	185.83±56.97	218.83±62.2	0.065
FBS (mg/dl)	89.2±12.55	175.93±55.01	0.00001
HbA1c	4.5±0.35	8.63±1.4	0.0005
MAU (mg/l)	16.56±5.53	22.65±7.75	0.00001

HDL: high-density lipoprotein, LDL: Low-Density Lipoprotein, FBS: Fasting Blood Sugar, HbA1c: Glycated haemoglobin, MAU: microalbumin in urine.

FBS & HA1c were significantly higher in both the diabetic individuals without retinopathy and diabetic individuals with retinopathy.

Table 3: SIRT1 gene expression levels & serum IL-17 levels in Diabetic & Control subjects

	Control, N=30	Diabetic cases, n=60 (diabetes without retinopathy + diabetes with retinopathy)	P value
PCR_CT	0.91 ± 0.75	0.61 ± 0.29	0.35
Serum IL17 (ng/ml)	5.69 ± 1.89	6.45 ± 1.75	0.055

PCR_CT: Polymerase Chain Reaction Cycle Threshold, IL17: nterleukin-17

When compared to the control group, the IL-17 levels raised in diabetic group. Our findings showed that SIRT1 gene expression was significantly reduced in DR cases compared to the control group. The SIRT1 gene expression levels were replicated using real-time PCR. Compared to the controls (0.91±0.75 fold), SIRT1 mRNA expression levels were lower in the RD (0.61 ± 0.29 fold) cases.

Association between gene expression level of *SIRT1* & presence of 4 SNPs in heathy:

The SIRT1 gene was found using real-time PCR, & the four SNPs were genotyped in PBMC samples obtained from 30 DR patients and 30 healthy controls. We investigated the connection between genotyping & gene expression. Nonetheless, there was no descriminate difference in the gene expression of the DR patients with different genotypes. We looked at the allelic expression of rs12778366, rs3758391, rs2273773, & rs4746720 in the healthy individuals and found significant differences for rs3758391. TC & CC were combined as there were only four CC homozygotes for rs3758391. The mRNA expression levels of SIRT1 in rs3758391 were substantially higher in 35 cases with the TT genotype (0.93±0.07 fold) than in 11 cases with the TC & CC genotypes (0.63 ± 0.07 fold, p = 0.006).

TC and CC were combined since there were only 4 CC homozygotes for rs3758391. However, the SIRT1 mRNA expression was not associated with the rs12778366 genotype (TT, 0.84 ± 0.06 fold, n = 32; TCCC, 0.88± 0.14 fold, n = 14; p = 0.80). SIRT1 expression levels were identical across all rs2273773 genotypes (TT, 0.79 ± 0.07 fold, n = 31; TC, 0.97± 0.11 fold, n = 12; CC, 0.61± 0.09 fold, n = 6; p = 0.29). In contrast, the genotypes of rs4746720 (TT, 0.62 ± 0.07 fold, n = 15; TC, 1.00± 0.09 fold, n = 25; CC, 0.77 ± 0.09 fold, n = 10; p = 0.07) did not correlate with the SIRT1 mRNA expression.

Table 4. Relationships between SIRT1 mRNA expression levels in DR patients & genotypes of rs12778366 rs3758391, rs2273773 & rs4746720

	TT	TC	CC	TC+CC
rs12778366	0.84	-	-	0.88
rs3758391	0.93	-	-	0.63
rs2273773	0.79	0.97	0.61	-
rs4746720	0.62	1.0	0.77	-

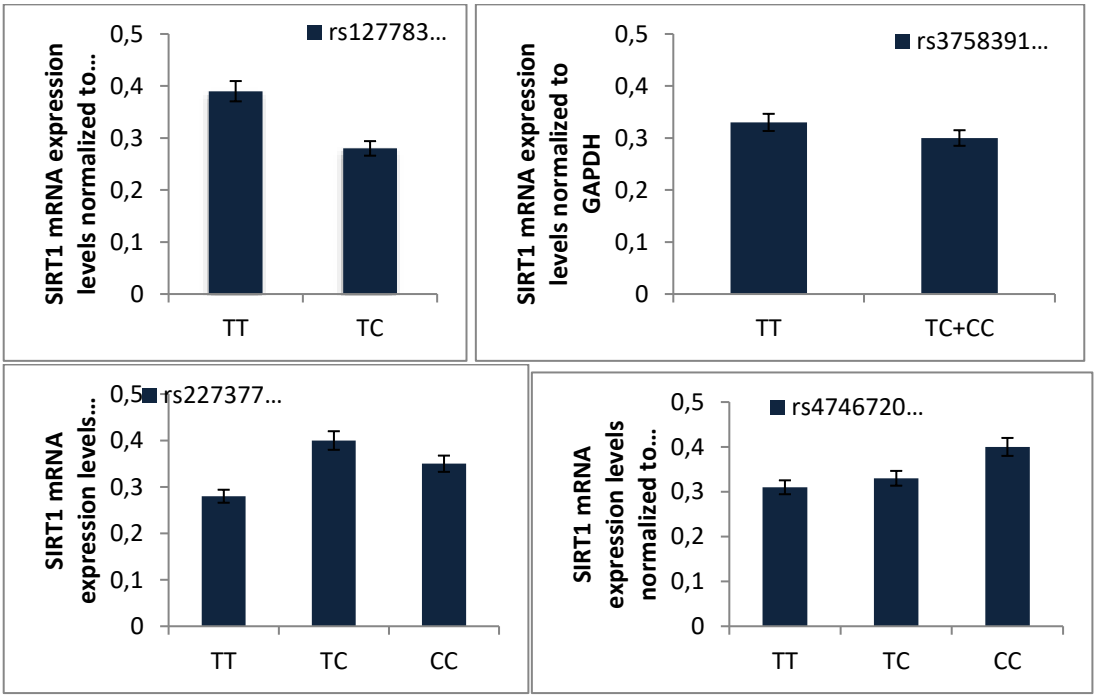

Figure 1. Relationships between the SIRT1 mRNA expression levels in DR patients & the genotypes

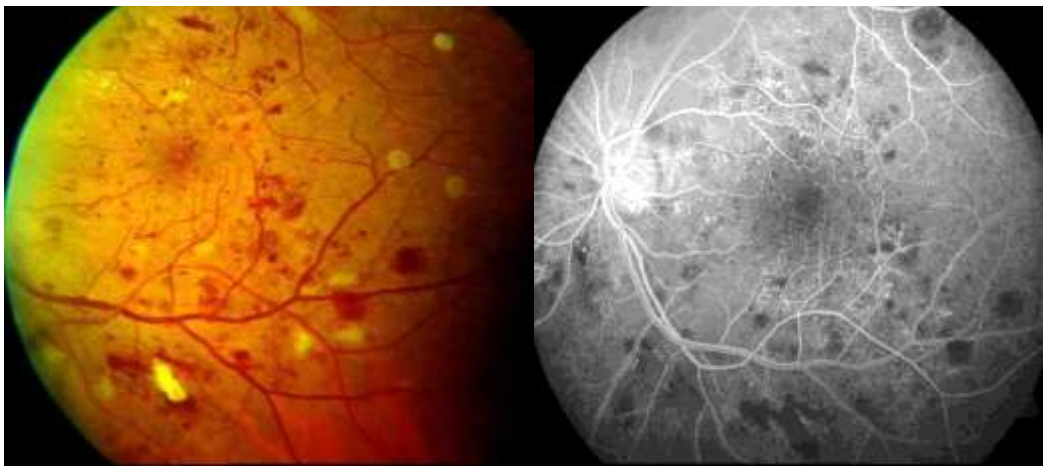

Figure 2: Fundus photo and fundus fluorescein angiography showing nonproliferative diabetic retinopathy

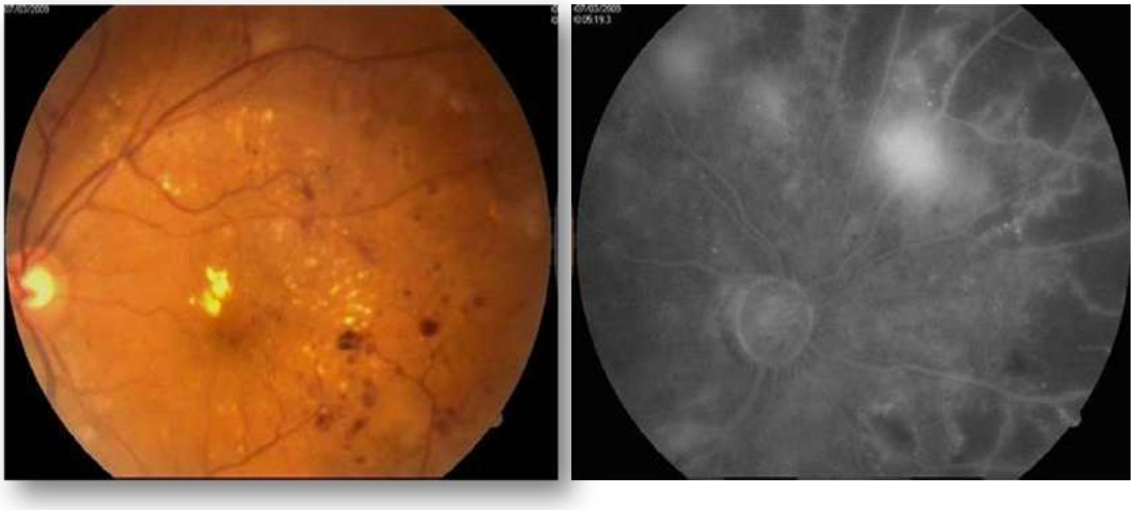

Figure 3: Clinical fundus picture of left eye showing nonproliferative diabetic retinopathy with Clinically-significant macular edema

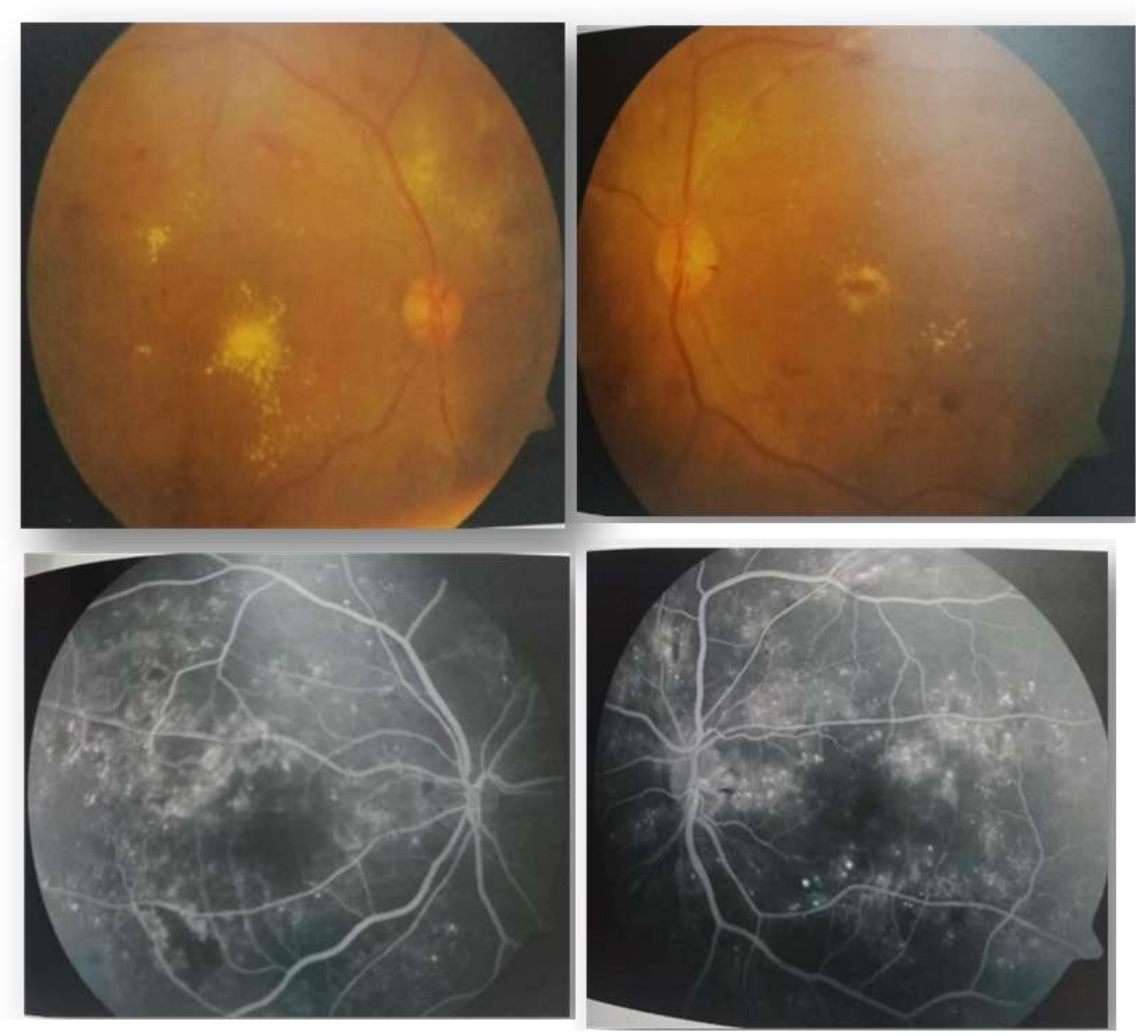

Figure 4: Showing diffuse exudative maculopathy (OU). Fundus fluorescein angiography showing diffuse leaks in and around macular area (OU)

Table 5: Basic demographic data in diabetic group between group 1(A) and group 1(B)

			Diabetes without retinopathy Group1(A) (n=30)	Diabetes with retinopathy Group1(B) (n=30)	P value
Age			56.2±7.5	57.2±6.5	0.45
Weight/kg			72.6±20.6	75.3±19.5	0.22
Height/cm			167.0±14.5	164.93±16.5	0.21
BMI			26.51±6.42	26.77±7.2	0.35
Diabetes duration			12.22±4.5	14.5±5.25	0.21
Sex	Female	N	16	16	1.0
		%	53.3%	53.3%	
	Male	N	14	14	
		%	46.7%	46.7%	
HTN	No	N	20	24	0.40
		%	66.7%	80.0%	
	Yes	N	10	6	
		%	33.3%	20.0%	
Total		N	30	30	
		%	100.0%	100.0%	

BMI: body mass index, HTN: hypertension

Table 6: LAB data in diabetic group between group 1(A) and 1(B)

	Diabetes without retinopathy Group1(A) (N=30)	**Diabetes with retinopathy Group1(B) (N=30)**	**P value**
Total cholesterol mg/dl	259.80±54.49	255.9±55.93	0.75
Triglycerides mg/dl	135.67±32.0	137.93±42.63	0.19
HDL cholesterol mg/dl	37.40±11.37	36.73±13.6	0.85
LDL cholesterol mg/dl	210.06±59.63	223.60±60.5	0.75
FBS mg/dl	174.60±64.5	180.26±58.63	0.55
HbA1c	8.60±1.23	9.01±1.15	0.021
MAU mg/l	21.40±6.87	23.9±9.14	0.02

HDL: high-density lipoprotein, LDL: Low-Density Lipoprotein, FBS: Fasting Blood Sugar, HbA1c: Glycated haemoglobin, MAU: microalbumin in urine.

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

Table 7: Laboratory variables in diabetic group between Diabetes with and without retinopathy Groups

	Diabetes without retinopathy Group1 (A) (N=30)	Diabetes with retinopathy Group1(B) (N=30)	P value
PCR/CT	0.60±0.27	0.62±0.30	0.012
IL17 ng/ml	6.44±1.65	6.49±1.88	0.022

PCR_CT: Polymerase Chain Reaction Cycle Threshold, IL17: nterleukin-17

IL17 was considerably greater (0.62±0.30 vs. 0.60±0.27, p=0.012) whereas SIRT1 gene expression was significantly lower in the Diabetes with Retinopathy Group than in the Diabetes without Retinopathy Group1.

Table 8. Correlation between SIRT1 mRNA expression levels and various Glycaemic levels

Glycaemic levels	SIRT_PCR_CT (r value)	P value
HbA1c ≤ 7.5	-0.326	0.09
HbA1c 7.5 to 9.0	- 0.448	0.12
HbA1c ≥ 9.0	- 0.245	0.004

PCR_CT: Polymerase Chain Reaction Cycle Threshold, HbA1c: Glycated haemoglobin

Different HbA1c levels were negatively correlated with SIRT1 mRNA expression levels. However, in diabetic patients, SIRT1 mRNA expression levels were highly correlated with HbA1c values greater than 9.0. SIRT1 mRNA expression levels and the regulation of glycaemic levels were significantly correlated, even after glycaemic levels were taken into account. SIRT1 gene expression decreases when HbA1c rises, indicating a negative connection between SIRT1 expression and HbA1c. This suggests that SIRT1 plays a protective function in preventing diabetic retinopathy ($r = -0.245$, $p = 0.004$).

Table 9: Correlation between gene expression and other parameters:

		PCR_CT
Age	r	-0.06
	P	0.75
Diabetes duration	r	-0.08
	P	0.61
Total cholesterol (mg/dl)	r	0.05
	P	0.69
Triglycerides (mg/dl)	r	0.28
	P	0.12
HDL cholesterol (mg/dl)	r	0.08
	P	0.59
LDL cholesterol mg/dl	r	-0.01
	P	0.85
HbA1c	r	-0.3
	P	0.07
IL-17 (ng/ml)	r	-0.33
	P	0.03
MAU levels (mg/l)	r	-0.12
	P	0.41
BMI	r	0.05
	P	0.69
FBS (mg/dl)	r	0.19
	P	0.20

BMI: body mass index, HDL: high-density lipoprotein, LDL: Low-Density Lipoprotein, FBS: Fasting Blood Sugar, HbA1c: Glycated haemoglobin, MAU: microalbumin in urine.

No significant correlation noted except significant negative correlation between PCR/CT & serum IL-17 levels

Table 10: The area under the Receiver Operating Characteristic curve (ROC), cutoff & validity of PCR/CT

Area	Cutoff	P	95% Confidence Interval		Sensitivity	Specificity
			Lower Bound	Upper Bound		
0.685	<0.52	0.006	0.598	0.895	82.0%	78.0%

PCR/CT: Polymerase Chain Reaction Cycle Threshold

Significant AUC of mRNA levels of SIRT1 cutoff <0.52 with 82.0%of sensitivity and 78.0% of specificity.

Table 11: The area under the Receiver Operating Characteristic (ROC) curve,, cutoff and validity of HbA1c

Area	Cutoff	P	95% Confidence Interval		Sensitivity	Specificity
			Lower Bound	Upper Bound		
0.789	>6.9	0.003*	0.675	0.989	82.0%	82.0%

Significant area under the Receiver Operating Characteristic curve (AUC) of HbA1c with cutoff >6.9 with sensitivity & specificity 82.0% & 82.0% respectively. SIRT1 expression & IL-6 gene expression had a negative correlation (r = 0.45, p = 0.0002).

DISCUSSION

Because of either insufficient insulin synthesis or a lack of cell sensitivity to its effect, diabetes mellitus (DM) is a chronic metabolic condition that is linked to abnormal blood glucose levels. It is regarded as a chronic illness that can impact the metabolism of fat, protein, and carbohydrates.[12] Diabetes is a rapidly expanding global epidemic brought on by ageing and fast-changing lifestyles, and it is predicted to become the leading cause of mortality in the ensuing decades.[13]

The most common neurovascular consequence of diabetes mellitus is diabetic retinopathy. The arteries that supply the retinal cells with oxygen and nutrients are weakened by the inflammatory consequences of hyperglycemia and dyslipidaemia. The damaged vessels start to leak, which causes hypoxia and retinal oedema. Growth factors including vascular endothelial growth factor (VEGF), which promotes the development of new blood vessels, are secreted by the retina in response to this breakdown. The newly formed vasculature are extremely fragile, prone to bleeding, and susceptible to blockage, which can worsen ischaemia.[14]

It has been established that SIRT1 is important for the development of DR.[15]At the ophthalmology outpatient department, a case control study was carried out. 30 non-diabetic, seemingly healthy individuals (Group II) were contrasted with 60 T2DM patients (Group I). Two further groups of diabetic patients have been created: 30 diabetic patients without retinopathy (Group IA) and 30 diabetic cases with retinopathy (Group I B). Age, sex, height, weight, and BMI were all well matched between the patient and control groups. RT-PCR was used to determine the levels of SIRT1 mRNA expression in the entire blood.

According to our findings, group I's SIRT1 gene expression was marginally lower than group II's. However, the data distribution across the two diabetes groups (Group IA and Group I B) revealed that the SIRT1 gene expression was considerably lower in the diabetic individuals with DR in Group I B.

In the current investigation, it was discovered that the PBMCs taken from DR cases had lower SIRT1 mRNA expression. We discovered a substantial correlation between rs3758391 and SIRT1 gene expression.

The SIRT1 mRNA expression level is linked to DR, as our case-control study showed, with DR patients' PBMCs exhibiting lower expression.

When we analysed the SIRT1 gene's mRNA expression levels in DR patients, we discovered that the DR patients had lower SIRT1 expression levels than the control group. Although no research has been done to explain this results, it appears that SIRT1 expression is more strongly associated with RD.

In our investigation, there was a negative correlation between the SIRT1 level and the IL-6 gene expression. SIRT1 knockdown has been shown to boost IL-6 expression, and DR patients have been found to have markedly higher IL-6 levels. These results suggest that one of the factors influencing the downregulation of SIRT1 gene expression is inflammation.

The consequences of the SIRT1 gene's non-coding genetic variation might be another contributing element. Non-coding genetic variations may affect gene expression by binding to distinct transcription factors, according to mounting evidence.[16]

In the current investigation, we found that rs3758391 and SIRT1 mRNA expression in healthy persons were strongly correlated, while rs3758391 was not in LD with the other three SNPs.

Additionally, it has been found that rs3758391 is linked to SIRT1 expression that is triggered by calorie restriction and may impact SIRT1 mRNA expression through the loss of p53 binding sites. The p53 consensus binding sequence's mirror-image symmetry is broken by the C allele of rs3758391.[17] Therefore, binding to p53 in vitro is reduced when a conserved region of the p53-binding element changes from T to C.

With regard to the harmful effects of p53, a protective negative feedback loop could be the cause of the high SIRT1 expression of the T allele of rs3758391.

Lastly, it has been discovered that SIRT1 deacetylates p53 and controls p53 activity in cells (18). In other words, by altering the p53 binding site, rs3758391 controls the expression of SIRT1. Therefore, the downregulation of SIRT1 gene expression may be influenced by genetic variations linked to SIRT1 and inflammation.

We also examined the relationship between the SIRT1 mRNA expression in the DR patients and the existence of the four functional SNPs; however, we were unable to identify any noteworthy variations between the various genotypes. The SIRT1 gene expression may have been impacted by the DR patients' usage of drugs before blood was drawn.

Although there were inconsistent findings for the alleles in relation to fasting glucose, BMI, and diastolic blood pressure, we did not find any significant relationships with SIRT1 mRNA expression in relation to rs2273773. rs2273773 is a synonymous mutation, however it can also act through noncoding activities either at the DNA or RNA levels before or after transcription. Through the inhibition of SIRT1 expression at the transcriptional level by peroxisome proliferator-activated receptor-γ (PPARγ), SIRT1 contributes to fat mobilisation (19). Thus, through PPARγ, Rs2273773 may have an impact on blood pressure and BMI.

Many regulatory proteins, including transcription factors connected to oxidative stress and apoptosis, become hyperacetylated when SIRT1 is

downregulated. Tu et al.[20] showed that SIRT1 expression was lower in DR animals compared to normal mice, which is consistent with our findings. The deacetylation of SIRT1 target genes Foxo1 and NF-κB, which prevented inflammation and oxidative stress, reduced the development of DR. Liu et al. reported the same findings.[21]

Song et al.[22] demonstrated that SIRT1 expression was inhibited in diabetic patient cells in T2DM participants. Al-Khaldi & Sultan[23] demonstrated that, in comparison to the control group, the SIRT1 mRNA expression level was significantly lower in those with a type 2 diabetes diagnosis. By preventing oxidative stress, these findings validated SIRT1's preventive function against diabetic vascular complications.[24] Variability in the results may be caused by variations in the number of cases chosen for each study, the environment, the diet, the patients' ethnicity, or other factors. Maloney et al.[25] demonstrated that SIRT1 expression was elevated, which runs counter to the current theory.

In our investigation, diabetics' serum IL-17 levels were marginally higher than those of the control group. According to Wang et al.[26] who demonstrated that IL-17A is actively involved in DR pathophysiology as it promotes retinal ganglion cell (RGC) death through mediation by retinal muller cells (RMC), IL17 was significantly higher in Group I B compared to Group IA. SIRT1 has been observed as a regulator of inflammatory reactions, namely through the inhibition of IL17 production.[27] Their PBMCs had lower levels of SIRT1 mRNA and protein expression. The findings showed a negative relationship between SIRT1 and IL-17, which may link SIRT1 to the development of DR. Hence, SIRT1 might be protective in DR.

In comparison to the healthy control group, our study showed a statistically significant increase in HbA1c in diabetes cohorts. Additionally, we found a statistically significant increase in HbA1c in the DR group compared to the diabetic group without retinopathy. Since hyperglycemia is the main risk factor for the onset and progression of DR, HbA1c is considered an essential biomarker of diabetes management.[28] According to our research, the DR group had higher HbA1c levels than the diabetic group without retinopathy, indicating that they are less well-managed and, hence, more vulnerable to diabetes-related issues, specifically DR.

In our study, the TC of diabetes patients was marginally higher than that of the control group, which appeared to be in good health ($P<0.127$). When compared to Group IA, TC increased insignificantly in Group IB ($p<0.897$). When comparing the diabetic group to the control group, LDL-c increased insignificantly ($P<0.068$), and when comparing Group IB and Group IA, it increased insignificantly ($p<0.828$). Compensatory hyperinsulinemia results from a reduction in normal insulin sensitivity in people with type 2 diabetes. This is attributable to the condition.

The presence of insulin resistance is linked to the activation of lipase in adipose tissue, which raises free fatty acids (FFAs). After travelling through the bloodstream and being absorbed by the liver, free fatty acids are esterified to create very low-density lipoproteins, which are then released back into the bloodstream. VLDL is converted to IDL and then to LDL in the blood vessels.[28] When comparing diabetic cases to healthy individuals, HDL-c fell insignificantly ($P<0.07$), and when comparing Group IB to Group IA, it reduced insignificantly ($p<0.903$). According to Song et al.[22] diabetics have higher levels of hepatic lipase expression, which impacts HDL-c. As a result, plasma HDL-c decreases because the kidneys catabolise the smaller HDL particles more quickly. Diabetics showed a statistically insignificant rise in TG compared to both group IB and the control group, but not to group IA. According to Rutledge et al.[29] the mechanism behind hypertriglyceridemia

may include delayed clearance of triglyceride-rich lipoproteins and increased hepatic production of VLDL. The main cause of this is higher concentrations of the substrates needed to produce triglycerides, namely glucose and free fatty acids. The small number of cases examined may be the cause of insignificant results.

The MAU level is significantly higher in the diabetic group compared to the control group in our study, and it is significantly higher in group IB compared to group IA. Our findings are supported by Ajoy-Mohan et al.[30] and Sobngwi et al.[31] who found that microalbuminuria is a sensitive indicator of early DR and a strong predictor of DR in type 2 diabetics. Compared to the controls (0.91±0.75 fold), SIRT1 mRNA expression levels were lower in the RD (0.61 ± 0.29 fold). There were no notable differences amongst DR patients with different genotypes. We looked at the allelic expression of rs12778366, rs3758391, rs2273773, and rs4746720 in the healthy controls and found significant differences for rs3758391.

When comparing the Diabetes with Retinopathy group to the Diabetes without Retinopathy Group1, SIRT1 Gene Expression was considerably lower, but IL17 was significantly greater (0.62±0.30 vs. 0.60±0.27, p=0.012).

Research indicates that RD patients' PBMCs have reduced SIRT1 mRNA levels. There was a negative association between SIRT1 expression and IL-6 gene expression (r = 0.45, p = 0.0002). Different HbA1c levels were negatively correlated with SIRT1 mRNA expression levels. However, in diabetic patients, SIRT1 mRNA expression levels were highly correlated with HbA1c values greater than 9.0. SIRT1 mRNA expression levels & the regulation of glycaemic levels were significantly correlated, even after glycaemic levels were taken into account. SIRT1 gene expression decreases when HbA1c rises, indicating a negative connection between SIRT1 expression and HbA1c. This suggests that SIRT1 plays a protective function in preventing diabetic retinopathy (r = -0.245, p = 0.004).

This study have several limitations. It is as a single-center study, the findings may not be generalizable to other population. The sampling method which may have introduced the selection bias, like severe cases. Study used only SIRT1 gene expression analysis, which may alone is not the biomarker involved in the molecular mechanism of diabetic retinopathy. Study lacked about the standardized criteria regarding the measurement of SIRT1 mRNA expression levels in diabetic retinopathy, which needs huge samples to be followed to establish a standardized operating procedure. Hence future research needs investigate to address these limitations and provide a more comprehensive understanding of retinal disease patterns in this population. The outcome of this study may useful to understand the molecular mechanism of disease progression and also setup the SIRT1 as a biomarker to measure the severity of the disease. However, future study needed to in-search of this new mechanism to establish as a new diagnostic biomarker and further to setup the treatment strategies.

CONCLUSION

The association between the SIRT1 gene expression levels and diabetic retinopathy was analysed in this study. The current research demonstrates that SIRT1 mRNA expression is decreased in diabetic retinopathy patients, indicating that SIRT1 is a gene will prevents the diabetic retinopathy for further progression. Furthermore, the identification of rs3758391 could be utilised as a biomarker for SIRT1-related disease prediction. To confirm the part these variations play in the pathophysiology of diabetic retinopathy, a more comprehensive functional investigation is necessary. In our study, IL17 serum levels were elevated and SIRT1 gene expression is

downregulated in diabetic retinopathy. SIRT1 may therefore be protective against the onset of diabetic retinopathy.

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